Combinaison de la modélisation biophysique et de marquages isotopiques pour estimer la connectivité démographique des populations marines : application à Dascyllus aruanus dans le lagon sud-ouest de Nouvelle-Calédonie

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Application à *Dascyllus aruanus* dans le lagon sud-ouest de Nouvelle-Calédonie

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Résumé

Comprendre la dynamique des populations marines est essentiel à une gestion efficace et requiert des connaissances sur la dispersion et la connectivité entre populations qui sont encore très lacunaires. Beaucoup d'organismes marins ont un cycle de vie bipartite avec une phase larvaire pélagique qui représente souvent la seule possibilité de dispersion. De nouvelles techniques de mesure de la dispersion larvaire, par marquage ou modélisation, ont été développées durant ces quinze dernières années. Cependant, les résultats de ces deux types d'approches ont rarement été comparés au sein d'un même système marin, limitant l'utilisation des modèles de dispersion dans les modèles de métapopulation. Dans cette thèse, nous utilisons ces deux types d'approches pour étudier la connectivité larvaire d'un poisson de récif corallien, *Dascyllus aruanus*, dans le lagon sud-ouest de Nouvelle-Calédonie. Notre modèle de dispersion montre que la rétention larvaire présente une variabilité temporelle élevée à l'échelle lagonaire et à l'échelle d'un patch de récif, et atteint périodiquement des valeurs élevées malgré des temps moyens de résidence courts. Le marquage artificiel transgénérationnel des otolithes montre des taux d'auto-recrutement relativement bas à l'échelle de la saison reproductive, suggérant une ouverture importante des populations, et une variabilité temporelle considérable de l'auto-recrutement. Enfin, les grandes différences entre les résultats du modèle et ceux des marquages appuient le besoin de mieux comprendre les processus qui facilitent la rétention larvaire comme les comportements de homing et la circulation des courants à très petite échelle.

Mots clefs

Dispersion larvaire, connectivité démographique, auto-recrutement, rétention locale, modélisation biophysique, marquage transgénérationnel, otolithe, *Dascyllus aruanus*, Nouvelle-Calédonie, récif corallien

Abstract

Understanding marine populations dynamics is critical to their effective management, and requires information on patterns of dispersal and connectivity that are still poorly known. Many marine organisms have a bipartite life history with a pelagic larval stage that often represents the only opportunity for dispersal. In the last decade, new empirical and simulation approaches to measuring larval dispersal have been developed, but results from these two different approaches have rarely been compared in the context of a single marine system, impeding the use of larval dispersal models in metapopulation models supporting decision making. In this doctoral research, we used both approaches to investigate larval connectivity for a coral reef fish, *Dascyllus aruanus*, in the South-West Lagoon of New Caledonia. Our biophysical dispersal model shows that larval retention exhibits considerable temporal variability at both lagoon and patch reef scales and periodically reaches large values despite low average water residence time. Artificial transgenerational marking of embryonic otoliths in the wild also showed relatively low self-recruitment rates indicating high population openness at the reproductive season scale, with considerable monthly variability of self-recruitment. Large quantitative discrepancies between simulations and empirical results emphasize the need to better understand processes that facilitate local retention, such as homing behavior and very small scale circulation patterns.

Key words

Larval dispersal, demographic connectivity, self-recruitement, local retention, biophysical modelling, transgenerational marking, otolith, *Dascyllus aruanus*, New Caledonia, coral reef

Table des matières

Table des matières vii

Liste des figures xi

Liste des tableaux xv

1 Introduction 1

2 *Dascyllus aruanus* as a biological model for studying larval dispersal 13
- 2.1 Introduction . . . 15
- 2.2 Benthic adult life . . . 16
  - 2.2.1 Habitat and home range . . . 16
  - 2.2.2 Feeding behavior and growth . . . 17
  - 2.2.3 Reproduction . . . 17
- 2.3 Pelagic larval life . . . 30
  - 2.3.1 Hatching . . . 31
  - 2.3.2 Planktonic larval duration . . . 31
  - 2.3.3 Swimming capabilities of planktonic larvae . . . 33
  - 2.3.4 Sensory mechanisms at settlement . . . 35
  - 2.3.5 Habitat and behavior at settlement . . . 37
- 2.4 Conclusion . . . 38

3 Wind-induced variability in larval retention in a coral reef system 41
- 3.1 Introduction . . . 44
- 3.2 Material and methods . . . 46
  - 3.2.1 Study area . . . 46

3.2.2 Local meteo-oceanography in summertime . . . 46
3.2.3 Study species . . . 48
3.2.4 Biophysical model . . . 49
3.2.5 Simulations . . . 49
3.2.6 Lagoon *vs.* reef retention . . . 50
3.2.7 Cross-correlations . . . 51
3.2.8 Larval settlement maps . . . 51
3.3 Results . . . 52
3.3.1 Time series . . . 52
3.3.2 Cross-correlations . . . 53
3.3.3 Settlement maps . . . 54
3.3.4 Sensitivity to release depth . . . 54
3.4 Discussion . . . 55
3.5 Acknowledgments . . . 58
3.6 Appendix A . . . 64
3.7 Appendix B . . . 65
3.8 Appendix C . . . 66

4 Evaluation of transgenerational isotope labeling of embryonic otoliths 69
4.1 Introduction . . . 72
4.2 Methods . . . 74
4.2.1 Study species . . . 74
4.2.2 Fish sampling and handling . . . 74
4.2.3 Enriched ^{137}Ba solution preparation . . . 75
4.2.4 Enriched ^{137}Ba injections . . . 75
4.2.5 Spawning success and larval rearing . . . 78
4.2.6 Otolith preparation and analysis . . . 78
4.2.7 Eggs and larvae measurements . . . 79
4.2.8 Statistical analysis . . . 80
4.3 Results . . . 81
4.3.1 Validation of Barium isotope markers . . . 81
4.3.2 Spawning success . . . 82
4.3.3 Size of 1-day eggs and 2-day larvae . . . 82
4.4 Discussion . . . 87
4.4.1 Success of maternal transmission . . . 87
4.4.2 Longevity in maternal transmission and repeated injections . . . 87
4.4.3 Impacts of marking on spawning success and condition of offspring . . . 88
4.4.4 Applications and implications for future studies . . . 89
4.5 Acknowledgments . . . 90
4.6 Supplementary material . . . 91

5 Monthly variability of self-recruitment for a coral reef damselfish 99
5.1 Introduction . . . 102
5.2 Methods . . . 103
5.2.1 Study species and area . . . 103
5.2.2 Transgenerational marking . . . 104
5.2.3 Settlers and juveniles sampling . . . 105
5.2.4 Otoliths analysis . . . 106
5.3 Results . . . 110
5.3.1 Self-recruitment and connectivity . . . 110
5.3.2 Settlement intensity and PLD . . . 112
5.4 Discussion . . . 112
5.5 Acknowledgments . . . 116
5.6 Supplementary material . . . 118

6 Comparison between a larval dispersal model and field observations 123
6.1 Introduction . . . 125
6.2 Methods . . . 126
6.2.1 Biophysical model . . . 126
6.2.2 Comparison between years . . . 126
6.2.3 Self-recruitment, total recruitment and connectivity . . . 130
6.3 Results . . . 132
6.3.1 Comparison between MARS3D versions . . . 132
6.3.2 Comparison between reproductive seasons . . . 133
6.3.3 Self-recruitment and total recruitment at the focal reef, and connectivity to the other reefs . . . 136
6.4 Discussion . . . 142
6.5 Appendix A . . . 146
6.6 Appendix B . . . 147
6.7 Appendix C . . . 148
6.8 Appendix D . . . 149
6.9 Appendix E . . . 150

7 Conclusion 153
7.1 Principaux résultats . . . 156
7.2 Perspectives . . . 160

Bibliographie 163

Communications 185

Contributions 191

Remerciements **195**

Liste des figures

1.1 Représentation schématique du concept de métapopulation. 3
1.2 Schéma d'un otolithe. 8
1.3 Localisation du lagon sud-ouest de Nouvelle-Calédonie. 10
1.4 Adulte *Dascyllus aruanus* dans une colonie de corail branchu. 11

2.1 Distribution map of *Dascyllus aruanus*. 15
2.2 *Dascyllus aruanus* life cycle. 16
2.3 Adults *Dascyllus aruanus* on their branching coral colony 17
2.4 Sampling area : the South-West Lagoon of New Caledonia (SWL). 19
2.5 Sex-change for *Dascyllus aruanus*. 20
2.6 Histological sections of *Dascyllus aruanus* gonads. 22
2.7 Weight-length relationship for *Dascyllus aruanus*. 24
2.8 Mean gonadosomatic index (GSI) measured for *Dascyllus aruanus*. 25
2.9 Mean number of *Dascyllus aruanus* settlers per colony. 25
2.10 First sexual maturity curve of *Dascyllus aruanus*. 26
2.11 Oocytes size frequency distribution for ten selected active *Dascyllus aruanus* females. 28
2.12 *Dascyllus aruanus* clutch. 28
2.13 Gonad weight-female length relationship for *Dascyllus aruanus*. 30
2.14 Otolith increment profile. 32
2.15 Estimated PLD for different settlement months for individuals collected on the central patch reef. 33
2.16 Estimated PLD for individuals collected on eight reefs surrounding the central patch reef. 34

3.1 Study area : the South-West Lagoon of New Caledonia. 47
3.2 Simulated retention rate time series, WRF wind speed and direction and probability of occurrence of weather regimes. 59
3.3 Cross-correlations between retention time series and along-shore wind component. 60
3.4 Cross-correlations between retention time series and weather regimes. . . 61
3.5 Maps of settlement using an 11-day precompetency period over the whole simulated period. 62
3.6 Maps of settlement using an 11-day precompetency period for simulations corresponding to hatching events followed by a precompetency period dominated at 75% by weather regime 1 or at 75% by weather regime 4. . . 62
3.7 Mean retention over the austral summer 2003-2004 at natal reef scale and at lagoon scale for different release depths 63
3.8 Maps of settlement using a 21-day precompetency period. 65
3.9 Location of the focal reef and the four reefs used to evaluate the impact of the focal reef choice on the results. 66

4.1 Aquarium experiment pictures. 75
4.2 $^{138}Ba/^{137}Ba$ ratios in otoliths of larvae produced by *D. aruanus* females injected once with $^{137}BaCl_2$. 84
4.3 $^{138}Ba/^{137}Ba$ ratios in otoliths of larvae produced by *D. aruanus* females injected monthly with $^{137}BaCl_2$. 85
4.4 1-day egg diameter and 2-day larval standard length for clutches produced at various times since the beginning of the experiment. 94

5.1 Study area. 104
5.2 Frequency histogram of Barium ratio measured in *Dascyllus aruanus* otoliths cores. 112
5.3 PLD of *Dascyllus aruanus* settlers, number of settlers per colony, and self-recruitment rate for each settlement month on the focal reef. 113
5.4 Time elapsed between settlement and collect in function of size at capture. 118
5.5 Profiles of $^{138}Ba/^{137}Ba$ ratio and $^{55}Mn/^{43}Ca$ ratio in otoliths of a *Dascyllus aruanus* control and a marked settler. 119

6.1 Study area : the South-West Lagoon of New Caledonia. 127
6.2 Retention time series. 135
6.3 Cross-correlations between retention time series and wind components. . 135
6.4 Average simulated value of self-recruitment at the focal reef obtained for increasing values of X. 138
6.5 Average simulated value of self-recruitment at the focal reef obtained for increasing values of P. 138

6.6 Combinations of parameters X and P. 139
6.7 Simulated time series of self-recruitment and total recruitment at the focal reef. 141
6.8 Habitat selection map for *Dascyllus aruanus* in the biophysical model. . . . 146
6.9 Simulated self-recruitment obtained for different release depth intervals. . 147
6.10 Number of focal reef's recruits that were released in each grid cell. 148
6.11 Mean gonadosomatic index measured for *Dascyllus aruanus* from February 2012 to January 2013. 150
6.12 Simulated total recruitment time series on the focal reef without and with GSI correction. 151

7.1 Trois types de populations structurées spatialement. 159

Liste des tableaux

2.1 Weight-length relationship for adults *Dascyllus aruanus* in New Caledonia. 18
2.2 Sampling dates of the *Dascyllus aruanus* colonies from which the length of the reproductive period was estimated. 19
2.3 Sex-ratio and functional sex-ratio for each collected *Dascyllus aruanus* colony. 21
2.4 Reproductive strategies of marine species. 27
2.5 Number of spawning events, spawning lag and batch frequency observed for each *Dascyllus aruanus* colony. 31
2.6 Review of studies that estimated PLD for *Dascyllus aruanus*. 32
2.7 P.values of t.tests performed between PLDs of different months estimated on the central patch reef. 33
2.8 P.values of t.tests between mean PLDs estimated on the eight surrounding reefs. 34

3.1 Description of the four weather regimes. 48
3.2 Statistical comparison between WRF model predicted values and observations at Amédée Lighthouse weather station. 64

4.1 Treatments used to evaluate mark success and effect of injections on spawning success and 1-day eggs and 2-day larvae size. 77
4.2 Operating conditions of the New Wave Research 213 nm UV laser and Agilent 7500cs inductively coupled plasma mass spectrometer. 79
4.3 Nonlinear models of enriched ^{137}Ba mark transmission success for *D. aruanus* females injected with single injections. 83
4.4 Generalized linear mixed-effects model of spawning success of *D. aruanus* females injected with single or repeated injections. 83

4.5 Number of spawning events, spawning lag and spawning periodicity observed for each colony. . . . 85
4.6 Linear mixed-effects models of 1-day eggs diameter and 2-day larval standard length produced by *D. aruanus* females injected with single or repeated injections. . . . 86
4.7 Fish weight, corresponding fork length and total length for *Dascyllus aruanus*, total length categories and injected volume of $BaCl_2$ diluted solution (ml) for each length category. . . . 93
4.8 Review of studies investigating transgenerational isotope labeling using injections of enriched ^{137}Ba. . . . 95

5.1 Injections dates of *Dascyllus aruanus* colonies on the focal reef, number of colonies injected each month and corresponding percentage of focal population marked. Settlement dates intervals and assigned settlement months. . . . 105
5.2 Number of *Dascyllus aruanus* marked settlers found on the focal reef and on the ten neighboring reefs in function of threshold value. . . . 109
5.3 Number of *Dascyllus aruanus* settlers collected on the focal reef and on the 10 neighboring reefs that were analyzed with LA-ICP-MS for each settlement month and presented a clear ^{55}Mn peak in otolith core, number of marked settlers with the default threshold of 5.76, fraction of settlers on reef j that originated from the focal reef and 95% confidence interval. . . . 111
5.4 Settlement estimation dates of *Dascyllus aruanus* on the focal reef, number of colonies sampled, number of settlers per colony and estimated total number of settlers for each sampling month on the focal reef. . . . 118
5.5 Operating conditions of the New Wave Research 213 nm UV laser and Agilent 7500cs inductively coupled plasma mass spectrometer. . . . 120
5.6 Details of the 13 *Dascyllus aruanus* settlers identified as marked. . . . 120
5.7 P.values of t.tests between mean *Dascyllus aruanus* PLDs. . . . 121
5.8 Number and percent of *Dascyllus aruanus* individuals measured each month on the injected colonies per size classes of total length. . . . 121

6.1 Details of the dispersal model runs. . . . 129
6.2 Comparison of retention values and dispersal distances between "old" and "new" versions of MARS3D. . . . 133
6.3 Comparison of retention values and dispersal distances between reproductive seasons. . . . 134
6.4 Simulated percentage of larvae exported from the focal reef to each settlement reef i. . . . 137
6.5 Same as table 6.4 for three different combinations of parameters X and P. . . . 140

6.6 Mean number of recruits estimated on the field and simulated with the model. 142
6.7 Habitat selection for *Dascyllus aruanus* in the biophysical model. 146
6.8 Simulated percentage of larvae exported from the focal reef to each settlement reef i. 149
6.9 Mean number of recruits at the focal reef estimated on the field and simulated with the model. 151

Chapitre 1

Introduction

La biosphère fait face à de multiples changements se produisant à une vitesse sans précédent : surexploitation, destruction d'habitats, invasions d'espèces, changement climatique. Ces changements ont souvent lieu à des échelles de temps trop courtes et/ou des échelles spatiales trop grandes pour permettre aux organismes de s'adapter à leur nouvel environnement par des mécanismes évolutifs (Clobert et al. 2012). Dans ce contexte, la persistance des populations d'êtres vivants est fortement remise en cause et dépend entre autres choses de notre capacité à mettre en oeuvre des mesures de gestion et de conservation efficaces permettant d'augmenter la résilience des écosystèmes i.e., leur aptitude à se rétablir suite à des perturbations (Nystrom et al. 2000). Cette capacité repose essentiellement sur notre compréhension de l'écologie des espèces et, en particulier, de leur dynamique en relation avec leur environnement.

Au sens écologique, une population désigne un ensemble d'individus de la même espèce vivant en interaction à un endroit particulier et à un temps donné. L'habitat est un endroit qui rassemble toutes les conditions permettant à cette espèce d'y effectuer une partie ou l'ensemble de son cycle de vie. En milieux terrestre et marin, l'habitat est très souvent discontinu. Ces discontinuités peuvent être dues à la nature même de l'habitat (e.g., patchs de récifs coralliens, sources hydrothermales, herbiers marins) ou résultent de perturbations naturelles (e.g., cyclone) ou anthropiques (e.g., déforestation, routes, barrages) (Fahrig 2003). La fragmentation de l'habitat se traduit par une séparation géographique des populations sur chaque fragment (ou patch) distinct d'habitat. Ces populations spatialement discrètes sont plus ou moins connectées entre elles par des échanges d'individus, formant des réseaux de populations appelés métapopulations (Hanski 1999, Sale et al. 2006, Cowen et al. 2007) (fig. 1.1). Le degré de connectivité entre sous-populations ou populations locales, et ses échelles spatiale et temporelle, sont au coeur du fonctionnement des métapopulations.

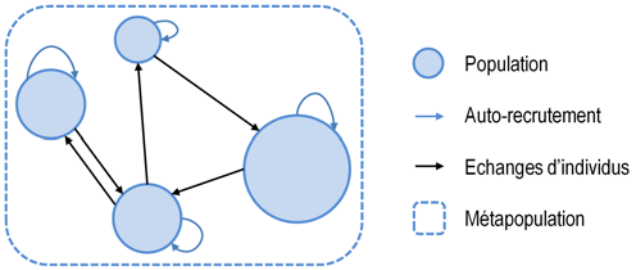

Figure 1.1 Représentation schématique du concept de métapopulation.

Les échanges d'individus entre populations locales peuvent intervenir à plusieurs moments du cycle de vie par dispersion de larves, mouvements de juvéniles ou d'adultes (Clobert et al. 2001). Il est important de faire la distinction entre deux types

de connectivité suivant l'échelle temporelle considérée. A l'échelle évolutive on parle de *connectivité génétique*, i.e., de flux de gènes entre populations qui ont lieu sur de nombreuses générations successives. Le degré de connectivité génétique entre populations détermine leur degré de différentiation génétique, et influence donc les phénomènes de spéciation et l'aire de répartition géographique des espèces (Sale et al. 2010). Il suffit de quelques individus échangés en moyenne par génération entre populations pour qu'elles soient génétiquement homogènes et donc génétiquement connectées. A l'échelle écologique on parle de *connectivité démographique*, i.e., d'échanges d'individus suffisamment importants pour influencer les paramètres démographiques des populations concernées (Sale et al. 2010). Les niveaux d'échanges requis pour influencer les populations sur le plan démographique sont bien supérieurs à ceux nécessaires pour maintenir l'homogénéité génétique entre populations (Planes et al. 2002, Cowen et al. 2007, Hedgecock et al. 2007). Le degré de connectivité démographique entre populations a un effet important sur leur dynamique et leur persistance à la fois à l'échelle locale et à l'échelle de la métapopulation (Hanski 2002, Hastings and Botsford 2006). La persistance d'une population est permise par le remplacement de ses individus : chaque adulte doit en moyenne être remplacé au cours de sa vie par un descendant lui-même capable de se reproduire. Dans le cas d'une métapopulation, la persistance du réseau de populations est possible si le niveau de connectivité démographique entre populations est tel que chaque adulte est remplacé par un nouvel individu capable de se reproduire, même si aucune de ces populations n'est auto-persistante (Burgess et al. 2014). Dans le cadre de mon travail de thèse je m'intéresse à la connectivité démographique des populations.

Dand le milieu marin, la grande majorité des organismes dispersent pendant leurs premiers stades de développement (Thorson 1950, Sale 1993). La dispersion des oeufs et des larves constitue même chez certains de ces organismes la seule occasion de déplacement au cours de leur cycle de vie (Armsworth et al. 2001, Kinlan and Gaines 2003). C'est le cas pour de nombreuses espèces démersales dont les stades de vie adulte sont relativement sédentaires, plus ou moins associés à un substrat benthique, et dont la phase larvaire s'effectue dans le milieu pélagique. Chez ces espèces la dispersion larvaire définit l'échelle spatiale de la connectivité (Cowen and Sponaugle 2009). Le stade larvaire correspond a un stade immature, de petite taille (généralement inférieur à 1 cm), très distinct du juvénile et de l'adulte d'un point de vue morphologique, physiologique et écologique (habitat, régime alimentaire), adapté à la vie pélagique (Kendall et al. 1984, Sale 1993) et soumis à des taux de mortalité très élevé (Leis 2006). Au cours de sa vie pélagique, la larve devient compétente en acquérant les capacités lui permettant de s'installer sur un habitat favorable (Jackson and Strathmann 1981) et de se métamorphoser en juvénile.

La dispersion larvaire peut être décomposée en trois étapes : le départ du site de ponte (sous forme de larve déjà éclose ou d'oeuf), le déplacement en pleine eau, et l'installation sur l'habitat juvénile ou adulte (Pineda et al. 2007). Plusieurs scénarii sont possibles après le départ de la larve du site de ponte : 1) la larve meurt pendant son déplacement (prédation, famine) ou à la fin de sa vie pélagique car elle ne trouve pas d'habitat favorable, 2) la larve revient à sa population natale (auto-recrue), 3) la larve s'installe sur une population distante (allo-recrue), et enfin 4) la larve s'installe sur un nouvel habitat (colonisation). La dispersion larvaire peut être influencée par divers facteurs. Le départ du site de ponte est dépendant de la reproduction : la saison, l'âge et la condition des adultes reproducteurs, le succès de fertilisation (Pineda et al. 2007). Le déplacement dans le milieu pélagique dépend d'une part de l'action des courants, qui soumettent les larves à des processus d'advection et de diffusion (Scheltema 1986), mais également de la durée de vie larvaire (quelques heures à quelques mois suivant les espèces, Shanks et al. (2003)), des capacités natatoires et comportementales des larves (Leis 2010), et de leur probabilité de survie qui est fonction de la disponibilité en nourriture et de la prédation. La combinaison de tous ces facteurs rend le déplacement dans le milieu pélagique extrêmement difficile à mesurer (Pineda et al. 2007). Enfin, l'installation des larves sur leur site d'arrivée dépend de la distribution et de la disponibilité de l'habitat ainsi que de leur comportement (Pineda et al. 2007).

Les populations marines ont longtemps été considérées comme des populations ouvertes, i.e., très connectées sur de grandes échelles spatiales (Caley et al. 1996, Roberts 1997). Cette représentation s'appuyait sur le paradigme selon lequel les larves dispersent de façon passive au gré des courants marins, à l'instar des graines de végétaux dispersées passivement par le vent en milieu terrestre (Clobert et al. 2012), pendant de longues périodes. L'observation de flux de gènes abondants entre populations et l'absence de liens entre la production locale de larves et l'installation à un site donné confortait cette théorie (Dixon et al. 1999). Le concept de métapopulations, utilisé en écologie terrestre depuis la fin des années 1960 (Levins 1969), était alors jugé inadapté aux problématiques marines (Sale et al. 2006). Cependant, depuis une quinzaine d'années, des études suggèrent une dispersion larvaire à plus petite échelle (1-100 km) et un retour des larves dans leur population natale plus fréquent que ce qui était admis jusqu'alors. Ainsi, Cowen et al. (2000) montrent à travers des simulations numériques basées sur les courants et le comportement larvaire que la rétention larvaire à petite échelle est possible. Ce résultat a été relayé par des mesures de terrain montrant un auto-recrutement très élevé (Jones et al. 1999, 2005, Almany et al. 2007, Planes et al. 2009) et une dispersion restreinte à quelques kilomètres seulement (Saenz-Agudelo et al. 2012). Parallèlement à ces études, la mise en évidence des capacités natatoires et sensorielles des larves a remis en cause le paradigme selon lequel la phase larvaire est

passive (Leis et al. 2011). Il est maintenant admis que les populations marines sont plus ou moins interconnectées au sein de métapopulations, et l'enjeu actuel est donc d'identifier la position des populations locales sur le continuum entre états ouverts et fermés (Bertness et al. 2001, Johnson 2005, Leis 2006, Jones et al. 2009). Quantifier l'échelle spatiale de la connectivité et identifier les facteurs la régulant (Leis 2006) est donc devenu un enjeu crucial pour pouvoir mettre en place des politiques de conservation et de gestion des populations marines adaptées à leur écologie (Kritzer and Sale 2004, Sale et al. 2005, Pineda et al. 2007, Jones et al. 2009, Leis et al. 2011). Par exemple, les connaissances sur la connectivité démographique sont un pré-requis essentiel à l'élaboration de réseaux d'aires marines protégées (AMPs) cohérents. Les AMPs sont principalement des outils de conservation de la biodiversité et de gestion des ressources halieutiques (Kritzer and Sale 2004, Leis 2006). Du point de vue de la conservation, le but des AMPs est d'assurer la persistance des populations en y régulant, voire en y interdisant, les activités humaines, à l'instar des réserves naturelles en milieu terrestre. D'un point de vue halieutique, l'objectif principal des AMPs est d'exporter, via la dispersion, des individus, issus de populations protégées et donc plus larges et plus fécondes, vers les populations exploitées. La capacité des AMPs à atteindre ces deux objectifs dépend de l'échelle de la dispersion et de la connectivité démographique entre populations (Palumbi 2003, Kritzer and Sale 2004, Almany et al. 2009, Botsford et al. 2009, White et al. 2014, Burgess et al. 2014). Cependant, les connaissances sur la connectivité démographique sont encore très lacunaires à cause de la difficulté à mesurer la connectivité, par dispersion larvaire notamment.

Pour quantifier la connectivité démographique par dispersion larvaire entre deux populations A et B, il faut être capable d'identifier les larves parties de A qui se sont installées sur B. Les techniques classiques de suivi du déplacement des individus en milieu marin par marquage physique interne ou externe (e.g., marqueurs électroniques, Nielsen et al. (2009)) s'avèrent très difficiles voire impossibles à mettre en oeuvre lorsque les individus observés sont extrêmement nombreux et de très petite taille (Thorrold et al. 2002, Levin 2006). Pour surmonter les difficultés liées à la petitesse et l'abondance des larves, diverses méthodes ont été développées durant les quinze dernières années (Jones et al. 2009, Leis et al. 2011). Ces méthodes peuvent être regroupées en deux grandes catégories (Kool et al. 2013) : les méthodes utilisant des marqueurs génétiques ou chimiques, et les méthodes de modélisation numérique de la dispersion larvaire.

Les outils classiques de génétique des populations permettent une estimation indirecte du nombre de migrants à partir de la variabilité des fréquences alléliques entre populations. Lorsque les différences génétiques entre populations sont faibles, cette approche n'est pas assez sensible pour estimer la connectivité démographique

(Sale et al. 2010). D'autres approches ont donc été développées, basées sur l'assignation d'individus à leur population d'origine ou à leurs parents et mettant en oeuvre des marqueurs génétiques ou chimiques.

Les méthodes génétiques permettant une évaluation de la connectivité démographique par assignation d'individus à leur population d'origine (tests d'assignation génétique) ou à leurs parents (analyses de parenté) mettent en jeu des marqueurs génétiques très polymorphes et spécifiques à l'espèce étudiée (e.g., les marqueurs microsatellites, Manel et al. (2005)). Les tests d'assignation génétique permettent d'affecter un individu à sa population d'origine en se basant sur la fréquence attendue de son génotype à plusieurs loci. Les deux principales limites de cette approche sont que : (1) toutes les populations d'origine potentielles doivent être échantillonnées et, (2) ces populations doivent être suffisamment différentiées génétiquement (leur connectivité démographique doit être faible) (Saenz-Agudelo et al. 2009). La plupart des populations marines ne remplissent pas ce dernier critère (Hedgecock et al. 2007). Les analyses de parenté permettent quant à elle d'assigner un individu à un de ces parents ou à un couple en sélectionnant le parent le plus probable parmi un groupe de parents potentiels. Les deux principales limites de cette approche sont que : (1) la localisation des parents au moment de la reproduction doit être connue si l'on souhaite connaitre la population locale d'origine (donc cette approche n'est pas adaptée pour des espèces mobiles au stade adulte) et, (2) la plupart des parents potentiels doivent être échantillonnés pour assurer une puissance de test statistique suffisante (Sale et al. 2010).

Les méthodes chimiques sont basées sur la propriété qu'ont certains tissus calcifiés comme les écailles, les coquilles, les otolithes ou les statolithes à incorporer et archiver quotidiennement les éléments chimiques de l'eau environnante au cours de la croissance des individus. Ces structures calcifiées sont métaboliquement inertes, si bien qu'une fois intégrés, les éléments sont retenus de façon permanente (Campana and Neilson 1985). L'analyse de la composition chimique naturelle des pièces calcifiées permet donc de mesurer la connectivité entre habitats suffisamment différenciés sur le plan chimique. Cette approche est utilisée fréquemment chez les poissons téléostéens car ceux-ci possèdent des otolithes (fig. 1.2), un tissu calcifié inerte de l'oreille interne qui enregistre l'âge, la croissance, et l'environnement chimique des individus (Campana 1999). Lorsque la chimie naturelle n'est pas assez discriminante, les techniques de marquage artificiel des pièces calcifiées peuvent être employées. Un marqueur chimique artificiel est incorporé dans les pièces calcifiées en modifiant l'environnement chimique des individus pendant la phase embryonnaire. Le marquage peut être réalisé par immersion des embryons dans une solution contenant le marqueur (e.g., marqueur fluorescent, Jones et al. (1999, 2005)). Cependant, ces techniques par

immersion nécessitent de manipuler les embryons *ex situ* et de pouvoir les collecter dans le milieu naturel ce qui s'avère très compliqué pour les espèces qui pondent en pleine eau et pas sur un substrat. Pour pallier ces difficultés, Thorrold et al. (2006) ont développé une méthode de marquage de masse, dit transgénérationnel, permettant un marquage *in situ* des embryons. Cette méthode repose sur la modification de l'environnement chimique des embryons par injection du marqueur dans la cavité abdominale des femelles pendant leur période de reproduction. Thorrold et al. (2006) utilisent une solution enrichie en un isotope stable du baryum, le ^{137}Ba. Les ions de baryum ayant approximativement le même rayon que le calcium, leur incorporation dans la matrice calcaire de l'otolithe est rendue possible par substitution avec les ions calcium (Campana 1999). La marque est détectée par ablation laser du noyau de l'otolithe et par analyse au spectromètre de masse du rapport isotopique ^{138}Ba/^{137}Ba, qui est plus faible que le rapport isotopique naturel pour les individus marqués (Thorrold et al. 2006).

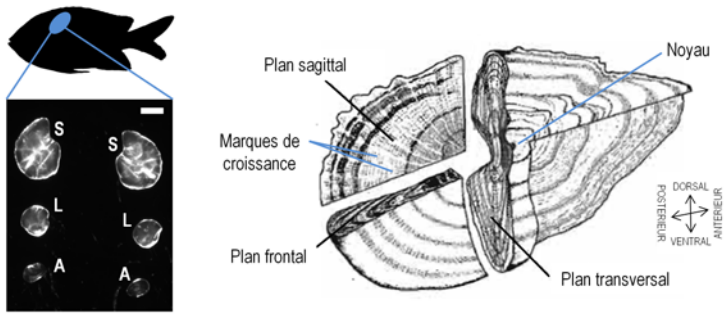

FIGURE 1.2 A gauche : Les trois paires d'otolithes d'un juvénile de *Dascyllus aruanus*. S : *sagitta*, L : *lapillus*, A : *astericus*. Barre d'échelle = 200 µm. A droite : Section à travers un *sagitta* montrant les trois plans d'orientation typiques et indiquant des marques de croissance et la localisation du noyau (modifié d'après Panfili et al. (2002)).

Les techniques de marquage fournissent un aperçu localisé et instantané de la connectivité larvaire. Même si des études récentes tentent d'estimer la variabilité spatiale et temporelle de cette connectivité (Saenz-Agudelo et al. 2012, Hogan et al. 2012), ces approches restent coûteuses et lourdes à mettre en oeuvre. Les modèles numériques de dispersion larvaire permettent de quantifier la connectivité en simulant la dispersion larvaire à différentes échelles spatiales et temporelles. Ils s'appuient sur des champs de courants issus de modèles hydrodynamiques en trois dimensions et peuvent prendre en compte la biologie et le comportement larvaire en incluant des processus tels que la migration verticale (Paris et al. 2007), la nage orientée (Staaterman et al. 2012), la mortalité (Cowen et al. 2000) et/ou la croissance des individus (Kone et al. 2013). La plupart de ces modèles biophysiques sont individu-centrés. Ils reposent

sur des algorithmes lagrangiens permettant le suivi de trajectoires individuelles de particules larvaires dans le domaine d'étude. A partir du moment où l'hydrodynamisme de la zone d'étude et les paramètres biologiques et comportementaux de l'espèce d'intérêt sont connus, les modèles biophysiques permettent de quantifier la connectivité larvaire, d'inférer la présence ou le rôle d'un mécanisme particulier et de générer des hypothèses (Miller 2007). Ils peuvent également être utilisés pour effectuer des projections dans le temps et évaluer par exemple les effets du changement climatique sur la connectivité larvaire simulée (Aiken et al. 2011, Brochier et al. 2013, Andrello et al. 2014). Comparativement aux approches empiriques de marquage, l'approche par modélisation est relativement peu coûteuse. L'augmentation des puissances de calcul a permis de faire des progrès considérables dans la modélisation des courants marins en permettant d'affiner la résolution spatiale des modèles hydrodynamiques. Si la partie physique des modèles biophysiques est relativement générique, la partie biologique est spécifique et donc difficilement généralisable. Les modèles biophysiques sont donc principalement limités par le manque de connaissances approfondies des processus biologiques (e.g., la croissance, la mortalité) et comportementaux (e.g., les distances de détection de l'habitat d'installation, Wright et al. (2011)) disponibles sur les larves. Ces modèles sont également très peu confrontés aux données de terrain issues des approches empiriques de connectivité, ce qui est pourtant indispensable pour pouvoir utiliser les résultats issus de ces modèles en appui aux décisions de gestion (Bowler and Benton 2005, Pineda et al. 2007, Leis et al. 2011).

Dans ce contexte, l'objectif de mon travail de thèse est de confronter deux approches complémentaires : une approche par modélisation biophysique de la dispersion larvaire et une approche par marquage isotopique transgénérationnel, pour étudier la connectivité démographique des populations de poissons dans le lagon sud-ouest de Nouvelle-Calédonie, en m'appuyant sur un poisson demoiselle, *Dascyllus aruanus*.

La Nouvelle-Calédonie est un archipel du Pacifique Sud constituant un des trois systèmes récifaux les plus vastes du monde (Andréfouët et al. 2009). Les deux tiers des lagons néo-calédoniens ont été inscrits au patrimoine mondial de l'UNESCO en 2008. La proximité de l'archipel indo-malais, considéré comme un point chaud de biodiversité, fait de la Nouvelle-Calédonie une des régions les plus riches en espèces marines. Mon travail de thèse se focalise sur le lagon sud-ouest de Nouvelle-Calédonie (fig. 1.3). La proximité de la ville de Nouméa et les fortes pressions anthropiques qui en résultent ont entraîné la mise en place progressive d'un réseau d'AMPs depuis le début des années 1980, et font du lagon sud-ouest une des zones lagonaires les plus étudiées de Nouvelle-Calédonie. La nature et la structure spatiale de la mosaïque d'habitat récifal y sont très bien documentées (Andréfouët and Torres-Pulliza 2004). La circulation océanique à l'intérieur du lagon a fait l'objet de nombreuses études basées

sur des modèles hydrodynamiques (Douillet 1998, Jouon et al. 2006, Ouillon et al. 2010, Fuchs et al. 2012). Tous ces paramètres font du lagon sud-ouest de Nouvelle-Calédonie un système idéal pour l'étude de la connectivité des populations par dispersion larvaire. Quelques études existent sur les flux de gènes (Planes et al. 1998) et sur les déplacements ontogéniques (Mellin et al. 2007, Paillon et al. 2014) de certaines espèces dans les lagons néo-calédoniens, et un modèle de dispersion larvaire passive a été mis en place à l'extérieur du lagon à l'échelle de la zone économique exclusive de Nouvelle-Calédonie pour le thon germon (Andres Vega pers. com.). Par contre, aucune étude ne s'est encore focalisée sur la connectivité démographique par dispersion larvaire à l'intérieur des lagons néo-calédoniens, et en particulier dans le lagon sud-ouest.

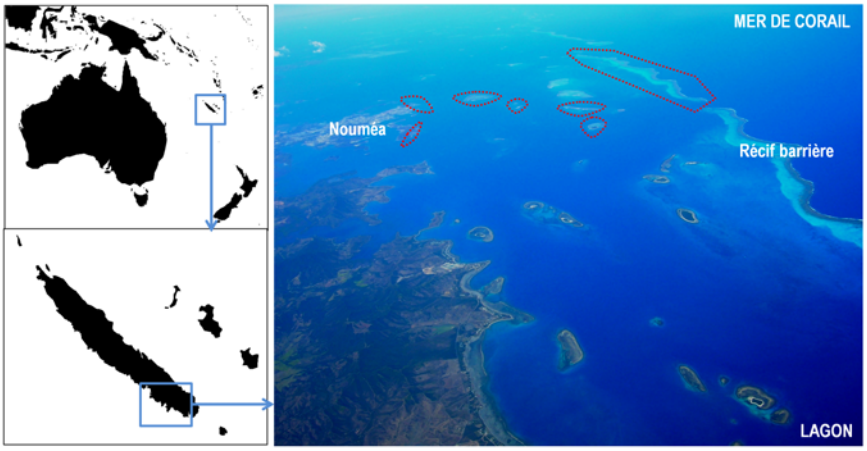

FIGURE 1.3 Localisation du lagon sud-ouest de Nouvelle-Calédonie. Le lagon s'étend sur une surface de 2000 km^2 environ et est séparé de la mer de corail par un récif barrière entrecoupé de passes. Un réseau d'Aires Marines Protégées a été mis en place, représenté ici en pointillés rouges.

Dans le **chapitre 2** je présente mon espèce d'étude : la demoiselle à queue blanche, *Dascyllus aruanus* (fig. 1.4). Ce chapitre est une synthèse bibliographique des connaissances actuelles sur la biologie et l'écologie de cette espèce, complétée par des connaissances acquises au cours de stages que j'ai co-encadré pendant ma thèse, notamment sur la reproduction de l'espèce et son stade larvaire.

Les trois chapitres suivants (3 à 5) sont présentés sous forme d'articles scientifiques.

Dans le **chapitre 3**, j'utilise un modèle biophysique pour simuler la dispersion larvaire de *D. aruanus* dans le lagon sud-ouest de Nouvelle-Calédonie. Je tente de répondre aux questions suivantes. La rétention larvaire est-elle possible à l'échelle

Figure 1.4 Adulte *Dascyllus aruanus* dans une colonie de corail branchu. Photo : A. Renaud.

du lagon et à la l'échelle d'un patch de récif malgré des temps de résidence courts des eaux lagonaires ? Quelle est l'influence de la date et de la profondeur de ponte, de la durée de la phase de pré-compétence, et d'un comportement de "homing" sur la rétention larvaire ? Des régimes de vent définis à l'échelle synoptique peuvent-ils expliquer en partie la variabilité de la rétention simulée à l'intérieur du lagon ? Dans ce chapitre, le modèle biophysique s'appuie sur un forçage hydrodynamique réaliste correspondant à une saison de reproduction (octobre 2003 à mars 2004) coïncidant avec une période neutre en termes d'épisodes El Niño et La Niña.

Le **chapitre 4** a pour objectif de valider la méthode de marquage trangénérationnel des larves de *D. aruanus* via injection des femelles avec une solution enrichie en ^{137}Ba en répondant aux questions suivantes. Quelles sont les doses de ^{137}Ba efficaces pour marquer les larves de *D. aruanus* par transmission maternelle ? Pendant combien de temps après l'injection la marque reste-t-elle transmise par les femelles à leurs embryons selon la dose ? Le succès reproducteur, la taille des oeufs et des larves sont-ils impactés par les différentes doses et par une répétition mensuelle des injections ? Pour répondre à ces questions, une expérience a été menée à l'Aquarium des Lagons de Nouméa sur la durée d'une saison de reproduction et une méthode inédite d'analyse microchimique des otolithes a été élaborée pour des larves âgées de 2 jours.

Le **chapitre 5** applique en milieu naturel la méthode de marquage validée dans le chapitre précédent pour répondre aux questions suivantes. Quelle est l'intensité et la variabilité temporelle de l'auto-recrutement pour une population locale de *D. aruanus* dans le lagon sud-ouest ? Quelles sont les distances de dispersion des larves et qu'elle

est la connectivité entre les populations locales du lagon sud-ouest ? Pour répondre à ces questions, des marquages ont été réalisés mensuellement sur deux périodes de reproduction d'une population focale située au centre du lagon sud-ouest, et des cohortes de recrues potentiellement marquées ont été collectées sur le récif focal et dix récifs alentours.

Le **chapitre 6** a pour but de comparer les résultats obtenus en milieu naturel et présentés dans le chapitre 5, aux résultats du modèle biophysique de dispersion présenté dans le chapitre 3, mais en simulant cette fois la dispersion larvaire pour l'année de terrain 2012. Il tente d'apporter des éléments permettant de comprendre les différences constatées entre les résultats des marquages et ceux du modèle biophysique.

Le **chapitre 7** synthétise et met en perspective les principaux résultats de ce travail de thèse.

Chapitre 2

Dascyllus aruanus as a biological model for studying larval dispersal

2.1 Introduction

Dascyllus aruanus (Linnaeus 1758) belongs to Pomacentridae (Perciformes) which contains over 200 tropical damselfish species (Allen 1991). Pomacentridae is one of the most intensively studied coral reef fish family and has contributed largely to general understanding of the biology and ecology of coral reef fish species (Leis 2006, Leis et al. 2011). Among damselfishes, the biology and ecology of *D. aruanus* have been particularly investigated for a number of reasons. First, this species is broadly distributed throughout the Indo-Pacific region from French Polynesia to Mozambique (fig. 2.1) and its strong dependence on corals and reef habitats make it an indicator of biodiversity and overall reef health (Chabanet et al. 2010, Pratchett et al. 2012). This species is very easy to identify, catch, and manipulate in the wild, and it adapts well to captivity which is of special interest for the ornamental industry (Wabnitz et al. 2003, Gopakumar et al. 2013, Rhyne et al. 2014). As most marine organisms, *D. aruanus* presents a bipartite life cycle, with a pelagic larval stage and a benthic adult stage (fig. 2.2). This species is very sedentary and territorial as an adult and is therefore a good model species to study marine population connectivity through larval dispersal.

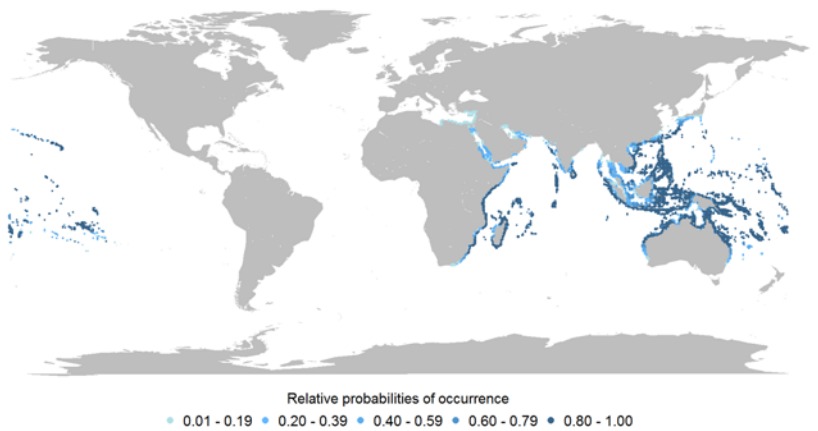

Figure 2.1 Distribution map of *Dascyllus aruanus* (plotted from relative probabilities of occurrence available in Kaschner et al. (2013)).

In this chapter I give an overview of biological and ecological knowledge of *D. aruanus* available in the literature, together with complementary information acquired throughout this thesis with a focus on reproduction, pelagic larval phase and settlement in the South-West Lagoon of New Caledonia (SWL). I first present information on the adult benthic life and follow with the pelagic larval life.

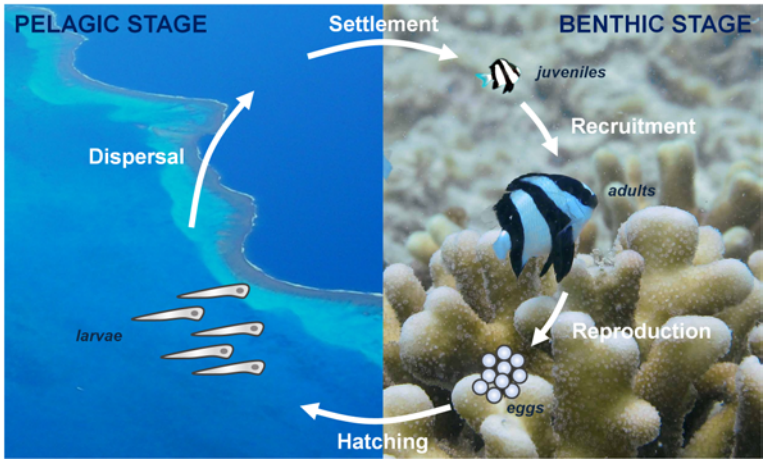

FIGURE 2.2 *Dascyllus aruanus* life cycle.

2.2 Benthic adult life

2.2.1 Habitat and home range

D. aruanus has a benthic sedentary and territorial adult stage. This species lives among live branching coral colonies (Coker et al. 2014) in spatially discrete groups with up to 80 individuals but typically less than 10 (Holbrook et al. 2000) (fig. 2.3). Live corals provide shelter to fish and fish enhance coral growth through defense from coral predators, aeration of coral tissue and nutrient provisioning (Chase et al. 2014). *D. aruanus* associates with over 20 coral species (Coker et al. 2014) and is more likely to occupy larger coral colonies than smaller ones (Nadler et al. 2013). Fish group size increases significantly with coral colony volume and with larger branch spacing (Sale 1972b, Holbrook et al. 2000). The association of *D. aruanus* with other fish species is common especially when branching density is low and coral size high (Nadler et al. 2013) (fig. 2.3).

The home range of *D. aruanus* is very limited usually centered on a single coral colony as fish restrict their activities to the water immediately around it (Sale 1971). Individuals have low potential to join a new group due to targeted and cooperative aggression by conspecific resident group members (Jordan et al. 2010, Coker et al. 2014). This aggressive behavior limits migration between colonies and has the effect of stabilizing group membership over time (Forrester 1991). Stable social groups are

organized into dominance hierarchies based on size whereby the largest individual in the colony is dominant to all other colony members (Forrester 1991, Jordan et al. 2010).

Holbrook et al. (2000) showed that, in the South Pacific, the availability of suitable habitat was generally an excellent predictor of *D. aruanus* density. However, Sale (1972b) indicated through observations on Heron reef on the Great Barrier Reef (Australia) that available suitable habitat might not be always fully used.

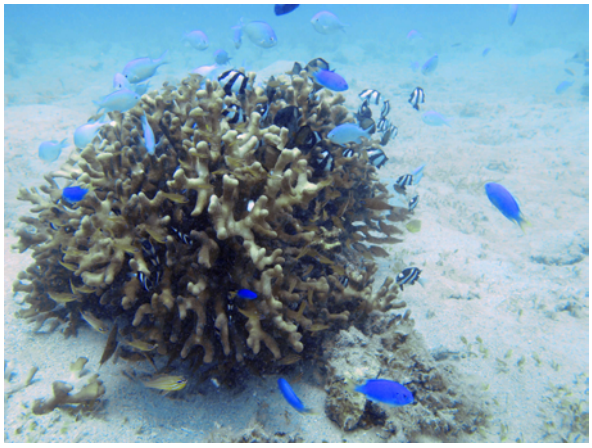

Figure 2.3 Adults *Dascyllus aruanus* on their branching coral colony living in association with other Pomacentridae (*Chromis viridis*, *D. reticulatus*, *Pomacentrus coelestis*) and Apogonidae (*Ostorhinchus rubrimaculata*) (photo by M. Cuif).

2.2.2 Feeding behavior and growth

D. aruanus is planktivore. Within social groups, *D. aruanus* gradually shifts its foraging tactics according to size : smaller fish feed on benthic prey such as isopods and copepods, and high ranked fish forage on planktonic copepods and larger-sized prey (Coates 1980a, Forrester 1991, Frédérich et al. 2010). In colonies with high fish density, *D. aruanus* shows higher specialization on prey size (Frédérich et al. 2010) and smaller fish feed at higher rates than larger ones (Forrester 1991). Three weight-length (W-L) relationships have been established for *D. aruanus* in New Caledonia (table 2.1).

2.2.3 Reproduction

Given the limited data on *D. aruanus* reproduction and its importance for the work planned in the thesis, I cosupervised a Master student who conducted a study in the SWL to complete existing knowledge.

TABLE 2.1 Weight-length relationship (Weight = a * FL^b) established for adults *Dascyllus aruanus* in New Caledonia. FL = fork length.

a	b	FL min (cm)	FL max (cm)	N fish	Reference
0.0716	2.635	2.4	6.5	105	Letourneur et al. (1998)
0.0415	2.989	2.4	6.5	112	Kulbicki et al. (2005)
0.0486	2.755	2.4	6.8	111	Paillon (2014)

Data collection in the SWL

Field survey

A first survey was conducted in February 2012 when thirteen reproductive colonies of *D. aruanus* comprising two to 89 fish were collected by scuba divers on the same day on a patch reef in the SWL (reef 8, fig. 2.4). Fish took refuge in their host coral when divers approached. For each colony, the branching coral was surrounded with a large plastic bag and fish were anesthetized with clove oil (20 to 40 ml at 5%), collected by hand, placed in a separate plastic bag to maintain colony integrity, and put immediately on ice after the dive. Back in the lab, every fish were measured to the nearest 0.1 mm total length (TL) using an electronic calliper. Gonads were extracted and placed in Bouin's fixative (71% of picric acid in distilled water solution ; 25% of formaldehyde ; 4% of glacial acetic acid) for every fish of the smallest colonies (6 colonies of less than 10 individuals and one colony of 20 individuals) and for a stratified random sample of fish for the biggest colonies (15 to 20 individuals per colony, respecting the size structure of the colony). A total of 103 gonads were extracted. After fixation, the 103 gonads were taken through a dehydration series, embedded in paraffin, and sectioned transversally using a microtome. Three to six sections (5 µm) were performed per sample. Sections were mounted on microscope slides, stained with hematoxylin and eosin, and viewed with an optical microscope to sex individuals and determine the most advanced gonad cells of each fish in order to classify them according to Cole (2002).

A second sampling was carried out over one year from February 2012 to January 2013 to determine the length of the reproductive period. One colony of approximately 10 mature individuals was collected monthly on a patch reef in the SWL (fig. 2.4, table 2.2). Colony collection, fish length measurements and gonads extractions were conducted as described in the previous paragraph. A total of 10 colonies was collected representing 130 fish. Gonads of each individual were also weighted to the nearest 0.1 mg with a precision balance.

Measurements in aquarium

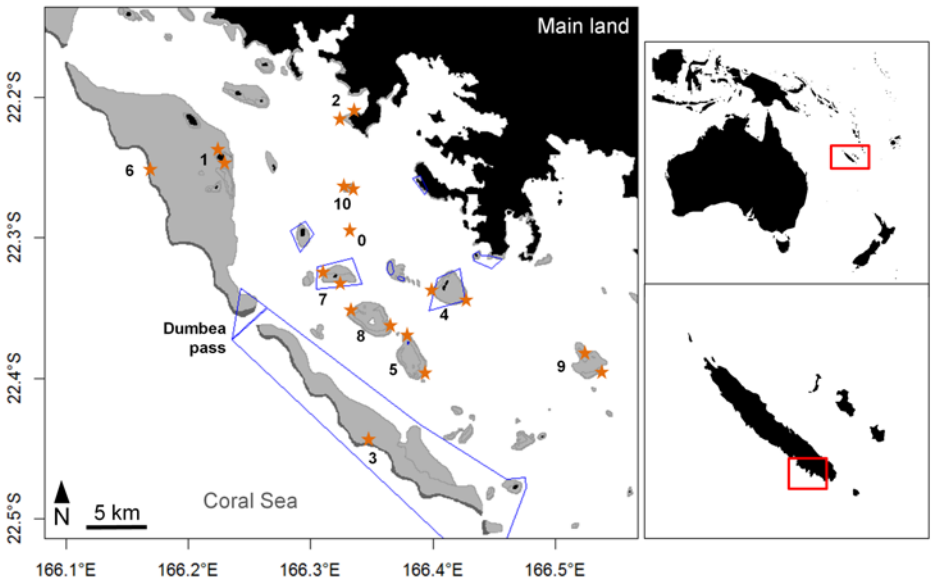

Figure 2.4 Sampling area : the South-West Lagoon of New Caledonia (SWL). Localization of the reefs (numbered from 0 to 10) and sampling stations (orange stars) where *Dascyllus aruanus* samplings were performed during the thesis. Land is depicted in black, barrier reef in dark grey and shallow reefs in light grey. Blue polygons localize Marine Protected Areas.

Table 2.2 Sampling dates of the 10 *Dascyllus aruanus* colonies from which the length of the reproductive period was estimated.

Colony	Sampling date	Reef n°
1	23/02/2012	8
2	06/04/2012	10
3	07/06/2012	0
4	26/07/2012	4
5	23/08/2012	7
6	21/09/2012	4
7	09/11/2012	4
8	22/11/2012	0
9	19/12/2012	4
10	17/01/2013	0

Another experiment was conducted in the aquarium facilities of the Aquarium des Lagons in Nouméa, New Caledonia, to measure parameters regarding *D. aruanus* reproduction. Twelve discrete colonies of *D. aruanus* were collected with their branching coral in the SWL in November 2011 (reef 8, fig. 2.4). The branching coral was surrounded with a large plastic bag, detached from the substrate, carried up to the

boat and placed in a seawater tub. The colonies were then transported to the aquarium facilities and the bigger individuals were selected so that each colony comprised approximately eight mature fish. Each colony was then transferred to 80 liters aquarium with its own branching coral. Spawning events were monitored daily.

Settlement survey

Settlement (mean number of settlers per colony) was estimated on reef 0 in the SWL (fig. 2.4) each month by counting the number of young settlers ($<$ 1.5 cm TL) on a random sample of colonies ($\sim$ 100 colonies each month) from October 2011 to May 2014.

Sex ratio

D. aruanus is a protogynous hermaphrodite (Coates 1982). Cole (2002) distinguished several types of individuals. Juveniles (Juv) with undifferentiated gonads become immature females (Fi), i.e., females with primary-growth stage oocytes only (inactive ovary) (fig. 2.5, fig. 2.6). Immature females become either active females (Fa, presence of oogenic ovary) that are able to spawn, or hermaphrodites (fig. 2.5, fig. 2.6). Hermaphrodites are either as inactive males (Hi, developing spermatogenic tissue and degenerating immature ovarian tissue), or as active males (Hma, spermatogenic testis and degenerating immature ovarian tissue), thus, being a stage of transition from female to male (Cole (2002), Asoh (2003), fig. 2.5, fig. 2.6). All individuals who become active males (Ma, presence of spermatogenic testis) pass through a female stage, but have never laid eggs (i.e., Fi to Hi transition) , although a sex-change from active females (Fa to Hi) cannot be totally excluded (Cole (2002), fig. 2.5, fig. 2.6).

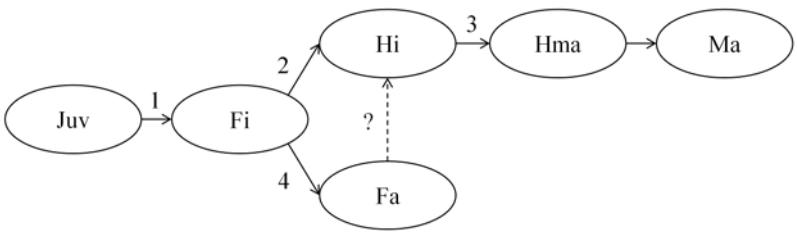

FIGURE 2.5 Sex-change for *Dascyllus aruanus*. Juv = Juvenile, Fi = immature female, Hi = inactive hermaphrodite, Fa = active female, Hma = active hermaphrodite male, Ma = active male. 1 : Developing ovarian tissue. 2 : Degeneration of immature ovarian tissue and development of spermatogenic tissue. 3 : Spermatogenesis. 4 : Development of vitellogenic oocytes. Adapted from Saulnier (2013).

Fricke and Holzberg (1974) showed that the sexual composition of *D. aruanus* colonies may change with the number of individuals. The sex ratio was female-biased for colonies of less than 6 individuals (a single-male and several females) and changed to unity as group size increased (Fricke and Holzberg 1974, Fricke 1977).

In the SWL, the sex ratio and functional sex ratio (i.e., based on active individuals only) were estimated for nine colonies collected in February 2012 (table 2.3) as :

$$\text{Sex ratio} = \frac{\text{Hi} + \text{Hma} + \text{Ma}}{\text{Fi} + \text{Fa}} \tag{2.1}$$

$$\text{Functional sex ratio} = \frac{\text{Hma} + \text{Ma}}{\text{Fa}} \tag{2.2}$$

The 3 smallest colonies of less than 6 fish contained one male only, in accordance with Fricke and Holzberg (1974) results. The number of males increased for bigger colonies, however the proportion of females (Fi + Fa) was always superior to the proportion of males (Ma + Hma) unless only active individuals were considered (e.g., colony 6). The sex ratio and the functional sex ratio were very similar (but see colony 6) because there were very few immature individuals.

Table 2.3 Sex-ratio and functional sex-ratio for each collected *Dascyllus aruanus* colony. N is the total number of individuals per colony. Numbers in brackets indicate the number of individuals for which maturity stage was estimated.

Colony	N	Fi	Hi	Fa	Ma + Hma	Sex ratio	Functional sex ratio
1	3	0	0	2	1	0.33 / 0.67	0.33 / 0.67
9	5	0	1	3	1	0.40 / 0.60	0.25 / 0.75
2	6	0	0	5	1	0.17 / 0.83	0.17 / 0.83
4	7 (5)	1	0	3	1	0.20 / 0.80	0.25 / 0.75
7	7 (6)	2	0	2	2	0.33 / 0.67	0.50 / 0.50
3	20	1	0	11	7	0.37 / 0.63	0.39 / 0.61
5	23 (14)	1	0	7	6	0.43 / 0.57	0.46 / 0.54
12	36 (12)	2	0	7	3	0.25 / 0.75	0.30 / 0.70
6	36 (19)	8	0	3	7	0.39 / 0.61	0.70 / 0.30

Reproductive seasonality

As for many species, spawning of *D. aruanus* is directly linked to water temperature. Therefore spawning seasonality varies significantly according to geographical position but the spawning peak takes place during the warm season. In Minicoy (India) for example, spawning events were observed all year long with a peak from April to January (Pillai et al. 1985). In Okinawa (Japan), spawning peaks between June and September (Mizushima et al. 2000).

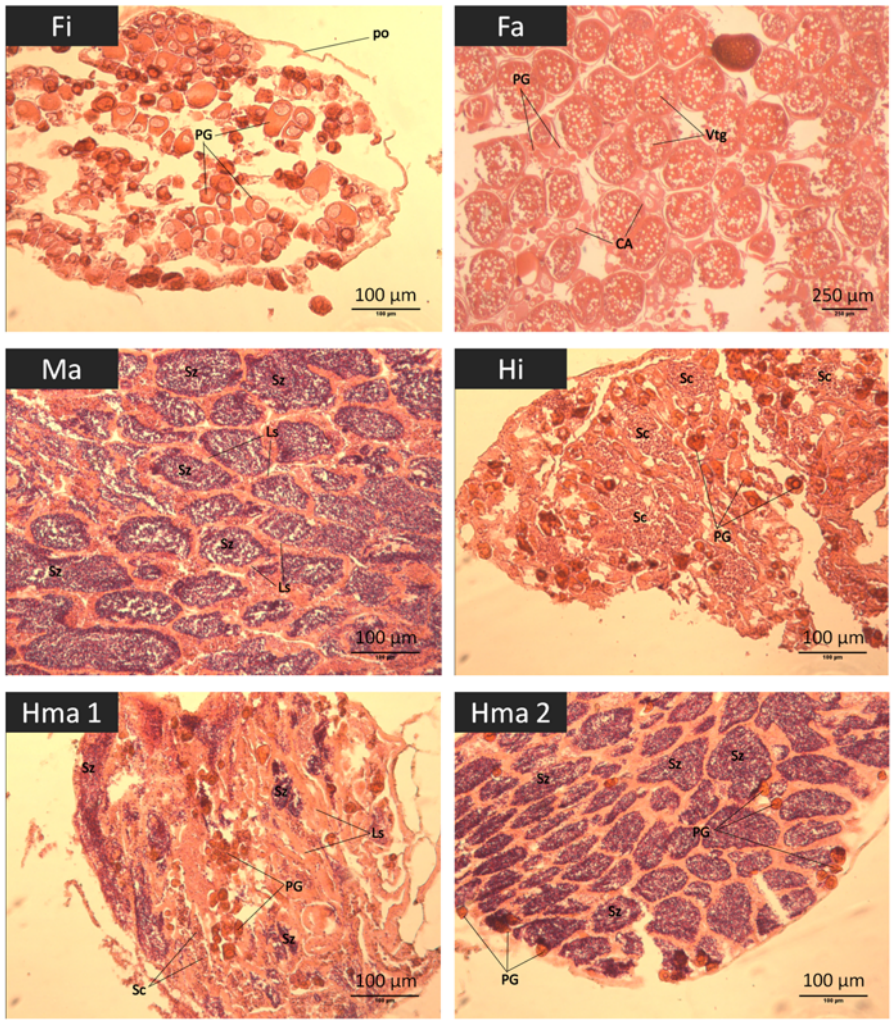

FIGURE 2.6 Histological sections of *Dascyllus aruanus* gonads. Fi : immature female (2.9 cm TL) with primary-growth stage oocytes (PG), po = ovarian wall. Fa : active female (4.8 cm TL) with primary-growth stage oocytes (PG), cortical alveolar phase oocytes (CA) and vitellogenic phase oocytes (Vtg). Ma : active male (4.5 cm TL) with spermatozoa (Sz), Ls = testis lobules walls. Hi : inactive hermaphrodite (4.6 cm TL) with primary-growth stage oocytes (PG) and spermatocytes (Sc). Hma 1 : active male hermaphrodite (4.6 cm TL) with primary-growth stage oocytes (PG), spermatocytes (Sc) and spermatozoa (Sz). Hma 2 : active male hermaphrodite (5.4 cm TL). Photos by E. Saulnier.

The length of the reproductive period in the SWL was determined based on the gonadosomatic index (GSI). GSI was calculated over one year for the 130 fish of the 10 colonies collected from February 2012 to January 2013 as :

$$\text{GSI} = \frac{\text{total gonad weight}}{\text{total body weight}} * 100 \tag{2.3}$$

and averaged over each colony. As the total body weight was not measured for all individuals, the W-L relationship was estimated for a sample of 79 fish including 48 females (2.89 to 5.73 cm TL) and 31 males and hermaphrodites (3.10 to 6.66 cm TL) collected between August 2012 and January 2013. The W-L relationship was described by :

$$\text{W} = a * \text{TL}^b \tag{2.4}$$

where W and TL are respectively the total body weight (g) and total length (cm) of the fish and a et b are parameters that were estimated with a non linear regression using the function *nls* under R-2.15.0 (http://www.R-project.org/). An analysis of covariance (ANCOVA) with a 95% confidence level was performed to determine if there were significant differences in the W-L relationship between sexes. Length and weight data were preliminary transformed to a natural logarithm function to satisfy assumptions of normality and homogeneity. ANCOVA showed that weight-at-length was not significantly different between sexes. Consequently the W-L relationship both sexes combined was estimated as W = 0.05802352 * $\text{TL}^{2.56508}$ with a regression value of 0.93 (fig. 2.7).

The mean gonadosomatic index (GSI) decreased from February 2012 (1.89 $\pm$ 1.69 SE) to June 2012 (0.34 $\pm$ 0.16 SE), stayed at low level until September 2012 (0.55 $\pm$ 0.19 SE), and then increased until reaching a peak from late November 2011 (4.40 $\pm$ 2.08 SE) to mid-December 2011 (4.37 $\pm$ 2.99 SE), before decreasing in January 2013 (3.33 $\pm$ 1.59 SE) (fig. 2.8). We concluded that the reproductive period of *D. aruanus* in the SWL lasts from October to March and peaks in late November - mid December. In accordance with this timing, settlement peaked each year around summer months January and February and was very low in winter between July and December (fig. 2.9). More surprisingly, there was also a peak in settlement around May or June each year, corresponding to spawning events occurring at the end of the warm season (fig. 2.9).

Size at maturity

The size of the smallest active females varies among studies : from 1.9 cm SL (standard length) ($\sim$ 2.3 cm TL) in Papua New Guinea (Cole 2002) to 3.0 cm TL in India (Pillai et al. 1985) and 3.0 cm SL ($\sim$ 3.7 cm TL) in Guam (Asoh 2003). The mean size at first maturity was estimated to be 3.8 cm TL in India (Pillai et al. 1985). Cole

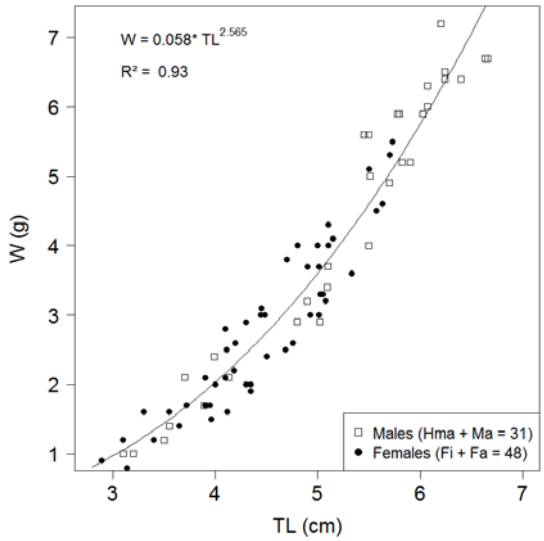

FIGURE 2.7 Weight-length relationship for *Dascyllus aruanus*. Males' data (white squares) include active males (Ma) and active male hermaphrodite (Hma). Females' data (black dots) include sexually inactive females (Fi) and active females (Fa). The black curve represents the best fitted power-law relationship between weight (W in g) and total length (TL in cm) for all individuals (n = 79 fish).

(2002) and Asoh (2003) reported that all individuals are sexually active above 3.9 cm SL ($\sim$ 4.8 cm TL).

All *D. aruanus* individuals collected in February 2012 in the SWL were sexually active above 4.6 cm TL. All individuals longer than 6.0 cm TL were males and 90% of the time the largest individual in a colony was a male. A sub-sample of 58 females (16 Fi and 42 Fa) was used to estimate female size at maturity. A logistic regression was used to fit a sigmoid curve to the proportion of mature females by length in the form :

$$\mathrm{P} = \frac{1}{1 + \exp(-(\alpha + \beta * \mathrm{TL}))} \tag{2.5}$$

where P is the probability that a female is mature at a given total length TL in centimeters, and α and β are parameters that define the shape of the fitted sigmoid curve. P was modelled under R-2.15.0 using the *glm* function with a logit link function and binomial error distribution. The predicted length at 50% maturity (Lm50) was calculated as :

$$\mathrm{Lm50} = \frac{-\alpha}{\beta} \tag{2.6}$$

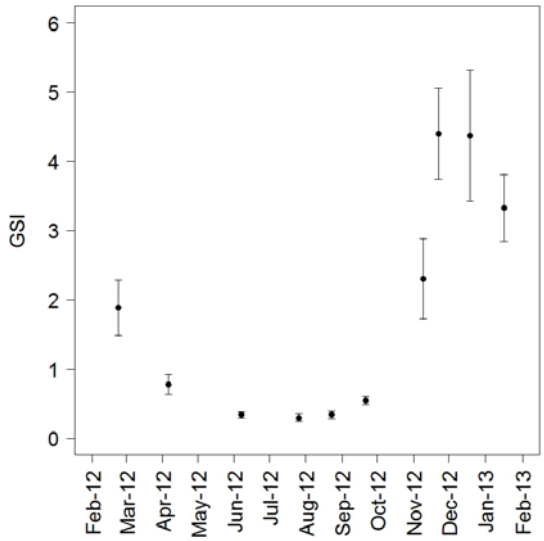

Figure 2.8 Mean gonadosomatic index (GSI) measured for *Dascyllus aruanus* from February 2012 to January 2013 (n = 130 fish). Error bars represents standard error.

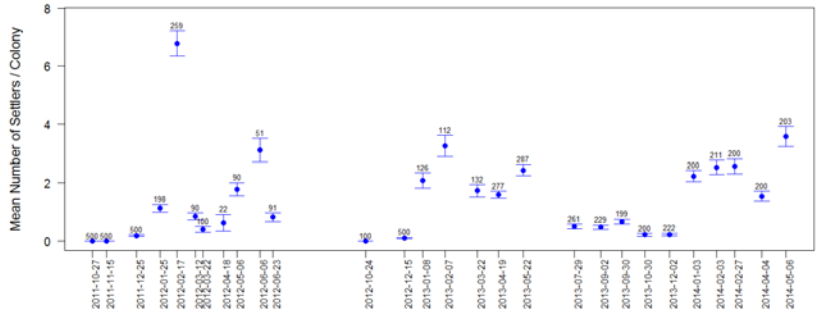

Figure 2.9 Mean number of *Dascyllus aruanus* settlers per colony estimated from October 2011 to May 2014. error bars represent standard error. Numbers above bars indicate the number of colonies examined to estimate settlement.

A confidence interval at 95% for Lm50 has been estimated by bootstrap. The estimated Lm50 of females was 3.75 cm TL [3.39 ; 4.07] (fig. 2.10). The smaller active female measured 3.10 cm TL.

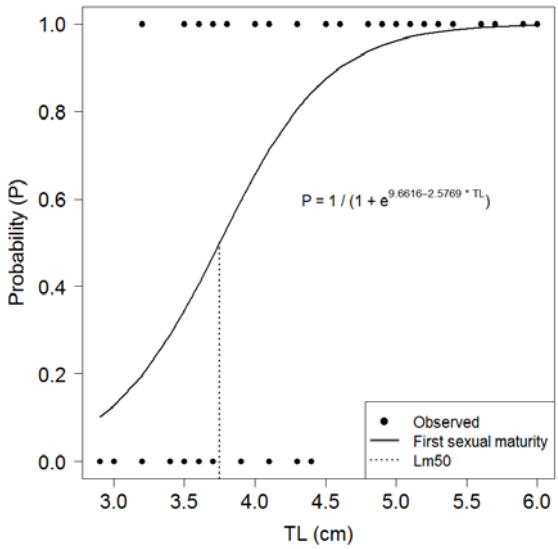

Figure 2.10 First sexual maturity curve of *Dascyllus aruanus* adjusted to the logistic model.

Fecundity

Gopakumar et al. (2009) observed spawning events of *D. aruanus* that occurred in captivity and found that the number of eggs laid by a single female ranged between 12,000 to 15,000. Pillai et al. (1985) counted the number of eggs present in an ovary at a time and found from 2,125 to 7,157 eggs per ovary (n = 5 females). Finally, Wong et al. (2012) collected egg clutches from 13 colonies in the wild (mean group size = 9.8 $\pm$ 1.65 SE) and counted the number of eggs under a dissecting microscope. Total clutch size ranged from 38 to 5,617 eggs (mean = 2,393 $\pm$ 475 eggs).

In order to estimate *D. aruanus* fecundity in the SWL, we had first to determine the species reproductive strategy (Murua et al. 2003) (table 2.4). As *D. aruanus* is iteroparous and a batch spawner species, its ovary development may be group-synchronous (oocytes of all stages are present in the ovary without dominant populations) or asynchronous (at least two cohorts of oocytes can be distinguished in the maturing ovary) (Murua et al. 2003). The usual method to determine if ovary development is synchronous or not is to estimate the oocyte size frequency distribution (an asynchronous ovary development leads to a continuous distribution of oocytes size frequency and a gap in this distribution is characteristic of a group-synchronous development, Murua et al. (2003)). When the ovary development is group-synchronous, fecundity

is determinate (the standing stock of yolked oocytes prior to the onset of spawning is considered to be equivalent to the potential annual fecundity, Murua et al. (2003)), otherwise fecundity may be determinate or indeterminate (potential annual fecundity is not fixed before the onset of spawning and unyolked oocytes continue to be matured and spawned during the spawning season, Murua et al. (2003)).

Table 2.4 Reproductive strategies of marine species.

	Ovary development	Fecundity	Type of spawning type
Semelparity	Synchronous	Determinate	Total
	Asynchronous		Batch
Iteroparity	Group-synchronous	Determinate	Total
			Batch
	Asynchronous	Determinate	Batch
		Indeterminate	

When the fecundity is determinate it is possible to estimate the annual fecundity (i.e., the total number of eggs released per female in a year) by counting, at the beginning of the spawning season, the number of oocytes destined to be spawned (Murua et al. 2003). Otherwise, annual fecundity may be estimated from other means, for example by using the mean number of eggs per spawning batch, the frequency of spawning events, the duration of the spawning season and the proportion of spawning females across the season (a function of GSI).

The oocyte size frequency of *D. aruanus* in the SWL was estimated from gonad sections of 10 active females (fig. 2.11). Pictures of ovary sections were taken with an optical microscope (LEICA DM 2000) and a Scion corporation for each female with a magnification of 40. Diameters of oocytes were measured on a randomly chosen picture for each female using the ObjectJ plug-in (`http://simon.bio.uva.nl/objectj/2-Tutorial.html`) of the ImageJ software (`http://rsbweb.nih.gov/ij/`). Only the entire and undamaged oocytes were measured totalizing 1,219 oocytes. The oocyte diameter was calculated as the average of the maximal and the minimal diameter.

The oocyte size frequency distribution was continuous with a peak around 35 µm (fig. 2.11), indicating an asynchronous ovary development. To determine if fecundity is determinate or indeterminate measuring other parameters such as the evolution of the number of advanced yolked oocytes or the incidence of atresia during the spawning season would have been necessary (Murua et al. 2003) but not achievable in the course of this work.

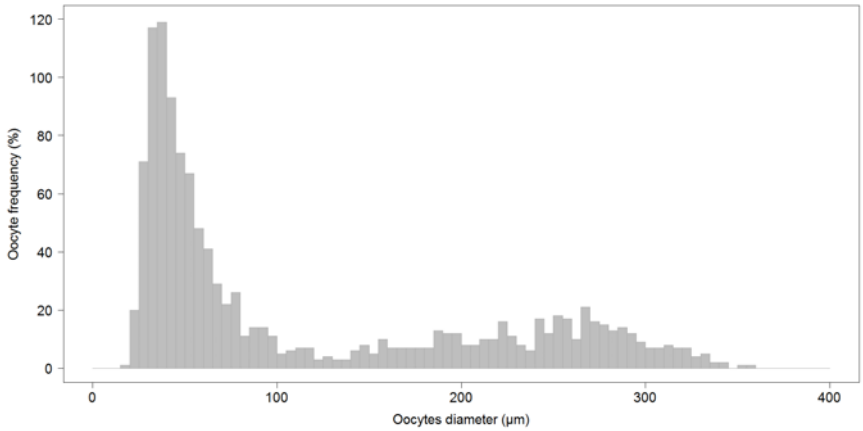

FIGURE 2.11 Oocytes size frequency distribution for ten selected active *Dascyllus aruanus* females.

Instead, we chose to estimate the annual fecundity through the batch fecundity (Murua et al. 2003) from the aquarium experiment. A total of 16 clutches corresponding to 16 distinct spawning events produced by nine colonies were pictured twice with a camera with a waterproof housing. The first picture covered the entire clutch and the second picture was zoomed and taken with macro mode (fig. 2.12).

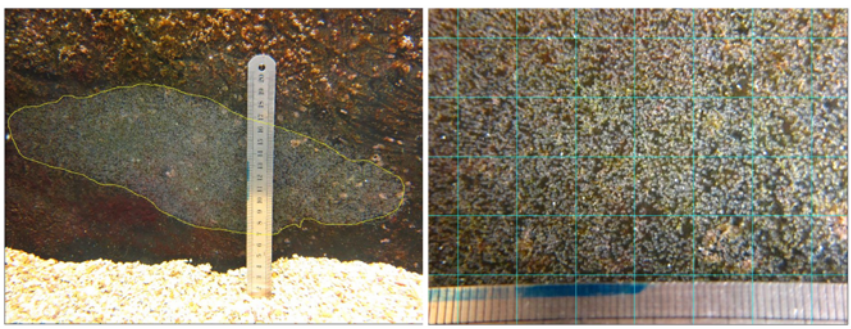

FIGURE 2.12 Left : *Dascyllus aruanus* clutch attached to a side of an aquarium. Right : Zoom on a clutch and 300 mm^2 squares used to count the eggs.

Pictures were analyzed using the ImageJ software. Total clutch area was measured on the first picture and egg density on the second picture using the ObjectJ plug-in and two to six randomly selected 300 mm^2 squares within which eggs were manually pointed and automatically counted. The total number of eggs per clutch was extrapolated

as :

$$\text{Number of eggs in the clutch} = \frac{N_1 + ... + N_n}{n * 300} * \text{Area} \tag{2.7}$$

where N_i is the number of eggs in the randomly selected square i, n is the total number of randomly selected squares in the clutch, and Area is the total area of the clutch (in mm^2).

This method resulted in a number of eggs per clutch varying from 5,829 to 46,217. As each clutch may correspond to several females we took into account the number of females per aquarium to finally estimate that *D. aruanus* in the SWL may spawn between 2,000 and 15,000 eggs per batch.

To estimate the variability of fecundity with size, a total of 29 gonads of females (Fi and Fa) of different sizes (ranging from 2.9 to 6.0 cm TL) collected in February 2012 were weighted to the nearest 0.1 mg with a precision balance. The relationship between gonad weight and female size was described as :

$$\text{Wg} = a * \text{TL}^b \tag{2.8}$$

where Wg and TL are the total gonad weight (mg) and total length (cm) of the fish, respectively, and a et b are parameters that have been estimated with a non linear regression using the *nls* function under R-2.15.0. We found an isometric relationship between gonad weight and female total length :

$$\text{Wg} = 1.099253 * \text{TL}^3 \tag{2.9}$$

showing that larger females have significantly larger gonads and are therefore likely to produce more eggs and/or bigger eggs than smaller females (fig. 2.13).

As *D. aruanus* is a batch spawner species, a final estimation of total egg production during the spawning period required to assess the number of batches. Females were shown to spawn several times at one week (Fricke and Holzberg 1974) to 2 months (Mizushima et al. 2000) intervals with an average periodicity of 2 weeks reported in captivity (Gopakumar et al. 2009). Spawning events are synchronous among a colony with all females spawning the same day or at a few days interval (Fricke and Holzberg 1974, Mizushima et al. 2000). A semilunar spawning cycle was observed in Okinawa with spawning occurring during a period of 2-4 days immediately before or around the time of the new and full moon, in the early morning (Mizushima et al. 2000, Gopakumar et al. 2009). Branching coral provide substrate for laying benthic eggs (Coates 1980a, Mizushima et al. 2000).

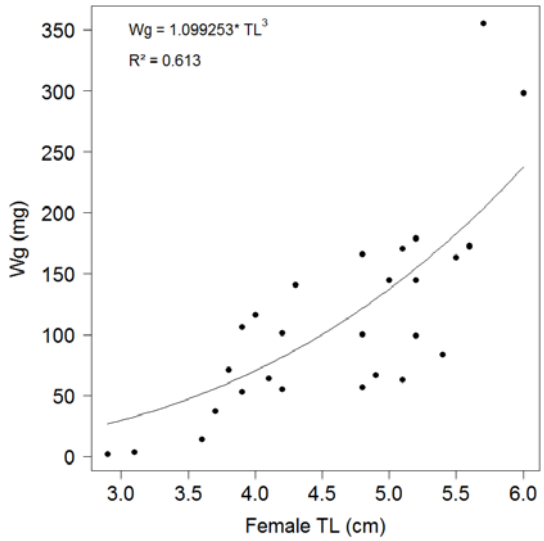

FIGURE 2.13 Gonad weight-female length relationship for *Dascyllus aruanus*. The black curve represents the best fitted relationship between gonad weight (Wg in mg) and female total length (TL in cm) for all individuals (n = 29).

In the SWL, we estimated batch frequency based on the aquarium experiment. Females of the same colony spawned synchronously and attached their eggs to sides of the tank making monitoring easy. Daily recording of spawning events started after one year of acclimation from October 2012 to January 2013 (for the 12 colonies) and lasted until April 2013 for the 2 colonies that were kept as control (i.e., not injected) in the transgenerational marking experiments (chapter 4). The batch frequency was calculated for each colony as the ratio of the total number of spawning events to the duration of the spawning period (table 2.5).

The mean batch frequency of the 12 colonies was 0.09 d^{-1} (standard deviation (SD) $\pm$ 0.03), i.e., a female produced one batch every 11 days on average (SD $\pm$ 3 days). Batch frequency may be over-estimated as we assumed that *all* females in one colony participated to *all* spawning events, which might not always be so.

2.3 Pelagic larval life

After the benthic adult life I now review the most relevant information on the pelagic larval life of *D. aruanus* from hatching to settlement.

Table 2.5 Number of spawning events, spawning lag (in days) and batch frequency observed for each *Dascyllus aruanus* colony.

Colony	Nb of spawning events	Total duration of spawning period (days)	Batch frequency (d^{-1})
1	16	158	0.10
2	15	132	0.11
3	4	50	0.08
4	9	80	0.11
5	7	67	0.10
6	6	109	0.06
7	6	55	0.11
8	10	67	0.15
9	5	77	0.06
10	5	65	0.08
11	6	78	0.08
12	7	76	0.09

2.3.1 Hatching

D. aruanus eggs hatch three days after spawning just after sunset (Danilowicz and Brown 1992, Mizushima et al. 2000, Gopakumar et al. 2009). The average length of newly hatched larvae is 2.4 mm (Gopakumar et al. 2009). The larvae are altricial type with no mouth opening at the time of hatching (Gopakumar et al. 2009). Gopakumar et al. (2009) observed mouth opening on the second day after hatching and a mouth gape of approximately 160 µm. Larvae start feeding from the third day after hatching (Gopakumar et al. 2009).

2.3.2 Planktonic larval duration

D. aruanus planktonic larval duration (PLD) has been estimated in several studies to last on average 3 weeks with a range extending from 2 to 4 weeks (table 2.6).

I estimated PLD for 579 *D. aruanus* individuals collected in the SWL including 466 individuals collected on a central patch reef (reef 0, fig. 2.4) over two reproductive seasons, and 113 individuals collected in March 2012 on eight surrounding reefs (fig. 2.4). I assumed that one increment corresponded to one day and that the first increment closest to the core of the otolith was formed at the time of hatching (Campana and Neilson 1985, Wellington and Victor 1989). PLD was determined for each fish as the age at which increment width abruptly fell (Wilson and McCormick 1999) (fig. 2.14). Settlement date of each fish was calculated as capture date minus number of days since settlement. Mean PLD were compared by pairs using t-tests with a 95% confidence level.

TABLE 2.6 Review of studies that estimated PLD for *Dascyllus aruanus*. SD is standard deviation and SE is standard error. N is the number of fish used for the estimate. GBR means Great Barrier Reef.

Mean PLD (days)	Error (SD or SE)	Range	N	Site	Reference
24.4	1.6 (SE)	20 - 28	5	One Tree Island (GBR)	Brothers et al. (1983)
20	2.4 (SD)	16 - 24	12	Palau	Wellington and Victor (1989)
22.4	NA	20 - 26	5	Lizard Island (GBR)	Thresher et al. (1989)
23.8	NA	23 - 25	4	Tuamotu (French Polynesia)	Lo-Yat (2002) in Juncker et al. (2007)
18.1	0.2 (SE)	14 - 23	122	Wallis island	Juncker et al. (2007)
26.3	1.7	24 - 29	10	Ryukyu Islands (Japan)	Soeparno et al. (2012)

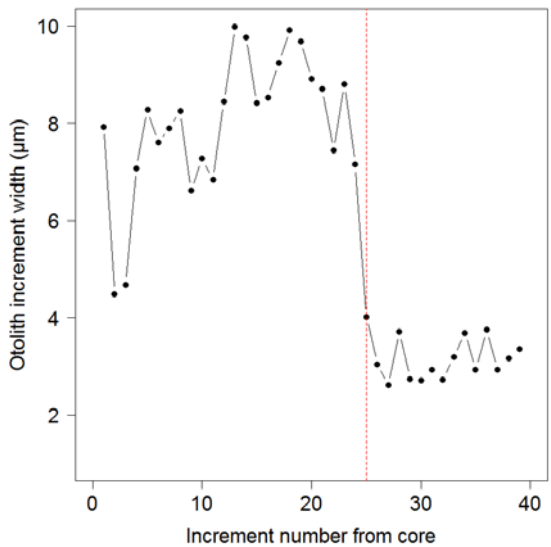

FIGURE 2.14 Otolith increment profile from longest axis plotted chronologically from otolith core for a *Dascyllus aruanus* individual collected in February 2013 (1.2 cm TL). Red vertical dotted line indicates PLD.

I found a mean PLD of 23.0 days (± 3.4 SD, range = 14 to 32 days, n = 466) on the central patch reef and there were significant differences in mean PLD between some months (fig. 2.15, table 2.7) with shorter PLD during months with warmer sea surface temperatures. Juncker et al. (2007) found similar seasonal variability of PLD for *D. aruanus* in Wallis island.

The 113 individuals collected in March 2012 on eight reefs were estimated to have all settled in February 2012. There seems to be a spatial pattern in PLD with shorter

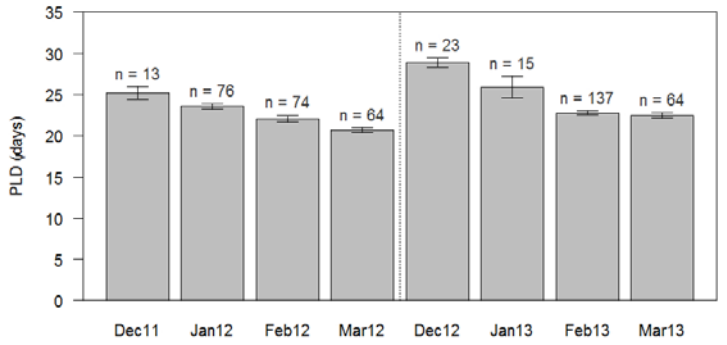

Figure 2.15 Estimated PLD for different settlement months for individuals collected on the central patch reef (reef 0, n = 466). Error bars represent standard error. The vertical dotted line separates the two reproductive seasons.

Table 2.7 P.values of t.tests performed between mean PLDs of different months estimated on the central patch reef (n = 466). Significant p.values (< 0.05) are indicated in bold.

	Dec11	Jan12	Feb12	Mar12	Dec12	Jan13	Feb13
Dec11	1	-	-	-	-	-	-
Jan12	0.0751	1	-	-	-	-	-
Feb12	**0.0029**	**0.0080**	1	-	-	-	-
Mar12	**8.44E-05**	**2.40E-10**	**3.80E-03**	1	-	-	-
Dec12	**1.17E-03**	**1.81E-09**	**2.72E-12**	**5.90E-14**	1	-	-
Jan13	0.6839	0.1088	**0.0146**	**1.42E-03**	**4.72E-02**	1	-
Feb13	**0.0124**	0.0670	0.1497	**5.90E-09**	**1.46E-10**	**0.0372**	1
Mar13	**0.0063**	**0.0269**	0.5316	**8.86E-05**	**1.53E-11**	**0.0237**	0.4163

PLDs for individuals settling North of the Dumbea Pass and longer PLD South of the pass (fig. 2.4, fig. 2.16, table 2.8). For example, average PLD of individuals collected on reef 3 and on reef 5 are significantly longer than PLD of individuals collected on the reefs 1, 2 and 10 that are located more in the North (fig. 2.4, fig. 2.16, table 2.8).

2.3.3 Swimming capabilities of planktonic larvae

In a review, Fisher (2005) showed that late-stage larvae (just before settlement) of a range of coral reef fish families were able to swim at speeds higher than the mean current speeds reported around most reefs. Leis and Carson-Ewart (1997) showed that, although *in situ* swimming speeds of Pomacentrids varied widely among species (7 to 35 cm s^{-1}), *D. aruanus* late-stage larvae were effective swimmers with a mean speed of 19.7 cm s^{-1} $\pm$ 1.9 SE (range = 9.1 to 30.1 cm s^{-1}) higher than the average current speeds

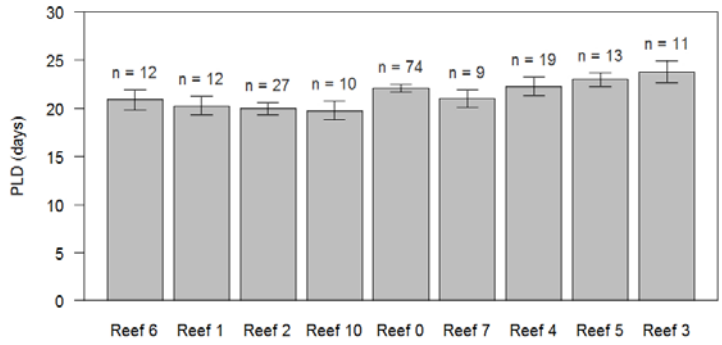

FIGURE 2.16 Estimated PLD for individuals collected on eight reefs (n = 113) surrounding the central patch reef (reef 0, n = 74) in March 2012. Error bars represent standard error. Reefs are plotted according to their localization from the North (reef 6) to the South (Reef 3) of the SWL (fig. 2.4).

TABLE 2.8 P.values of t.tests between mean PLDs estimated on the eight surrounding reefs (n = 113). Significant p.values (< 0.05) are indicated in bold.

	Reef 1	Reef 2	Reef 3	Reef 4	Reef 5	Reef 6	Reef 7	Reef 10
Reef 1	1	-	-	-	-	-	-	-
Reef 2	0.8586	1	-	-	-	-	-	-
Reef 3	**0.0274**	**0.0096**	1	-	-	-	-	-
Reef 4	0.1615	0.0667	0.3093	1	-	-	-	-
Reef 5	**0.0347**	**0.0038**	0.5452	0.5463	1	-	-	-
Reef 6	0.6464	0.4772	0.0718	0.3540	0.1107	1	-	-
Reef 7	0.5848	0.3994	0.0679	0.3565	0.1006	0.9524	1	-
Reef 10	0.7456	0.8371	**0.0132**	0.0827	**0.0139**	0.4335	0.3727	1
Reef 0	0.1029	**0.0087**	0.1819	0.8959	0.2929	0.2973	0.2869	**0.0423**

in their study area (10 to 15 cm s^{-1}). Fisher et al. (2005) also measured critical swimming speeds in captivity, by placing late-stage larvae in a swimming flume and increasing the current speed incrementally over time until the larva could no longer maintain its position for the full time interval (2 min in this study). The highest speed maintained is termed critical speed. Fisher et al. (2005) found an average critical swimming speed of 24 cm s^{-1} $\pm$ 0.8 SE, (range = 19.7 to 27.1 cm s^{-1}) for *D. aruanus* late-stage larvae (mean TL = 0.94 $\pm$ 0.02 cm SE), which is intermediate compared to other coral reef fish species (Leis 2006).

To determine the time larvae could maintain such an active swimming behavior, Stobutzki (1998) run an experiment in a swimming flume where fish were swum until they were fatigued, i.e., they could no longer maintain position against the current.

The time until they became fatigued was recorded and from this the travelled distance was calculated. They showed a significant correlation between sustained swimming ability and total length of individuals. They found that *D. aruanus* late-stage larvae (mean TL = 1.17 cm) were able to travel a distance of 21.37 km against a water speed of 13.5 cm s^{-1}. This suggests that speeds as high as 50% of critical swimming speed are sustainable by late-stage *D. aruanus* larvae for considerable time periods (over 24 h) (Fisher and Wilson 2004).

Fisher and Bellwood (2003) showed that the proportion of time larvae spent swimming at night increased rapidly towards the end of the larval phase for five coral reef species, including a Pomacentrid. In addition, they showed that the swimming speeds of larvae were significantly higher at night than during the day. Patterns of nocturnal activity appear to relate to the nocturnal settlement behavior of larvae.

All the above cited studies concentrated solely on the late pelagic phase. Fisher et al. (2000) suggested that some species have the potential to actively modify their dispersal patterns from an early age. Fisher (2005) suggested that the development of swimming ability can be described adequately as a linear increase from zero at hatching to a species-specific maximum at settlement. Calculations based on this developmental pattern suggest that most reef fish families could substantially influence their dispersal patterns relative to ocean currents for over 50% of their larval phase Fisher (2005). Leis (2006) underlined the fact that swimming abilities might develop sooner as expected, but suggested that it might not actually come into play until competency to settle is attained, or particular cues from settlement habitats are detected.

Active swimming behavior allows larvae to exert some control over their depth (Huebert 2012). Huebert and Sponaugle (2009) suggested that pressure is their primary proximate cue for vertical navigation. The depth at which larval fishes swim during their pelagic phase depends on the species and time of day (Leis et al. 2011), but the larvae of many species, including damselfish, are found at relatively shallow depths (Leis 1991, Hendriks et al. 2001). Irisson and Lecchini (2008) showed that Pomacentridae postflexion larvae tend to have a shallower depth distribution than flexion and preflexion larvae.

2.3.4 Sensory mechanisms at settlement

The potential influence of swimming capabilities on survival depends on the ability of larvae to orient in the open ocean (Paris et al. 2008, Leis et al. 2014) particularly towards their settlement habitat. Fishes larvae may use a range of sensory mechanisms effective over different spatial scales to detect and actively choose settlement sites,

including sound, smell and sight (Montgomery et al. 2001, Lecchini 2004, Lecchini et al. 2005).

Sound

The use of sound is the best understood mechanism (Leis et al. 2011). Sound travels well underwater with little attenuation, and is current-independent but location-dependent (Leis et al. 2011). Larvae of many coral reef fishes, and especially Pomacentridae, can hear as soon as otoliths are formed, i.e., during much of their pelagic larval phase and possibly before they hatch (Simpson et al. 2005). Settlement-stage larvae can distinguish among sounds (Leis et al. 2002, Radford et al. 2011) and are attracted to settlement habitats sounds (Simpson et al. 2004). Simpson et al. (2008) showed that coral reef fish larvae prefer the higher-frequency invertebrate-generated audible component of reef noise.

Distances over which larvae can detect a given sound vary among species and largely increase with ontogeny (Leis et al. 2011, Wright et al. 2011). Mann et al. (2007) suggested that larval fishes could not detect settlement habitat sound at distances superior to 1 km. But recent studies suggest otherwise (Leis et al. 2011, Wright et al. 2011) and that large reefs should be detectable from larger distances than small ones (Leis et al. 2011).

Smell

Olfactory cues are both current- and location-dependent (Leis et al. 2011). Larvae are able to detect odors during most of their pelagic phase and even during their embryonic stage (Arvedlund and Nielsen 1996, Atema et al. 2002, Kavanagh and Alford 2003, Dixson et al. 2014). Arvedlund and Nielsen (1996) showed that small post-settlement *D. aruanus* larvae had relatively high olfactory ability compared to other damselfish, suggesting good olfactory ability during younger stages. Pre-settlement larvae can detect odors associated to predators, conspecifics (Sweatman 1988), or water to which they were acclimated (Atema et al. 2002, Gerlach et al. 2007). The latter suggests that, once their settlement stage is reached, larvae may be able to return to their natal reef by actively swimming within the reef-odor plume. Little is known about the ontogeny of olfactory ability or the range over which larvae can localize odor sources (Leis et al. 2011). Lecchini et al. (2014) suggested that fish larvae could detect chemical cues emitted by coral reefs until 1 km. Paris et al. (2013) showed that pelagic reef fish larvae discriminated reef odor and responded by changing their swimming speed and direction. They concluded that reef fish larvae could smell the presence of

coral reefs from several kilometers offshore and that odor was a primary component of their navigational system by activating other directional sensory cues.

Sight

Celestial cues are current- and location-independent and can therefore apply over broader scales than other sensory abilities like sound and olfaction (Leis et al. 2011). Hawryshyn et al. (2003) reported that juveniles of damselfishes possessed the most complex polarization sensitivity recorded for any vertebrate. Mouritsen et al. (2013) showed that solar compass may be used for orientation in coral reef fish larvae. Although polarized light will not assist larvae to locate a particular reef, it can enable them maintain a general position, or to orient in a particular direction which is helpful in counteracting a prevailing current (Leis et al. 2011). Leis et al. (2011) suggested that celestial visual cues might be used early in development.

Other cues

To our knowledge, the use of magnetic fields for orientation has not been shown in marine fish larvae (Leis et al. 2011). Tide and wind have been suggested to be cues for orientation of pre-settlement spiny lobster larvae (Kough et al. 2014).

2.3.5 Habitat and behavior at settlement

D. aruanus larvae settle on branching coral colonies at varying depths (Leis et al. 2002). Jones (1997) showed that *D. aruanus* larvae settled preferentially to deeper sites (4 - 8 m depth). A growing number of studies show that coral reef fish larvae are able to recognize their natal reef through imprinting on chemical cues during embryonic stage, which could help explaining strong site attachment and high values of self-recruitment observed for many species (Atema et al. 2002, Gerlach et al. 2007, Dixson et al. 2008, 2014).

Settlement occurs at night for a range of coral reef fish families (Kingsford 2001). Accordingly, Sweatman (1985b) observed that a large proportion of *D. aruanus* larvae settled in darkness, when light levels were low and adults were hidden in the coral, suggesting that vision is unlikely to be important in habitat selection. Schmitt and Holbrook (1999) showed that settlement of *D. aruanus* in French Polynesia was following a semilunar cycle with settlement peaks lasting 3-5 days around the two quarter moon phases.

Late larvae of a few species of benthic fishes are known to school prior to settlement but there is limited information on when these larvae first begin schooling (Leis 2006). Genetic studies indicated that fish siblings settled together for several species (Planes et al. 2002, Selkoe et al. 2006, Bernardi et al. 2012), among which *D. aruanus* (Buston et al. 2009), suggesting that larvae may journey together throughout their entire planktonic dispersal phase from hatching to settlement. Kamel and Grosberg (2013) highlighted several factors that can promote the formation of dense conspecific aggregations during dispersal including gregarious larval behavior, consistent habitat preferences, variable settlement events and physical transport processes.

The presence of conspecifics appears to be a major cue for settlement in *D. aruanus* (Sweatman 1983, 1985a,b). Sweatman (1983) reported enhanced settlement of *D. aruanus* larvae on coral heads where conspecific fish predominate. Sweatman (1983, 1985a, 1988) and Jones (1988) found that the presence of *D. aruanus* increased the settlement of conspecifics and deterred the settlement of heterospecifics through a mechanism involving dissolved chemical cues (Sweatman 1988). Because adult and juvenile *D. aruanus* live in social groups and occupy the same habitat (Forrester 1990, 1991), Risk (1998) suggested that the presence of adults indicates suitable areas for settlers.

However, it has been observed that aggressive behavior of adults towards juveniles may force juveniles to settle in corals adjacent to an adult group until they reach a sufficient size to join this group (Sale 1972a, Booth 1992, Ben-Tzvi et al. 2012).

2.4 Conclusion

The bipartite life cycle of *D. aruanus*, with a sedentary territorial adult stage and a pelagic larval phase, makes this species adequate for larval connectivity studies as the larval phase is the only opportunity of dispersal. The broad repartition of *D. aruanus* throughout the Indo-Pacific (Kaschner et al. 2013) allows conducting comparative studies in a full range of different ecosystems. This species is also easy to identify, collect and manipulate, which are important criteria when conducting field experiments. As this species is a batch spawner that spawns with a high frequency during an extended reproductive season, it is possible to investigate the temporal variability of larval connectivity patterns. *D. aruanus* adapts well to captivity (Wabnitz et al. 2003) which is essential if experiments in aquarium are required. However, raising *D. aruanus* larvae is notoriously difficult, as for many other tropical reef fish larvae due to their typically small size, small mouth gape, and simple digestive system at the time of first feeding (Moorhead and Zeng 2010) leading to problems of starter feed (Gopakumar et al. 2009). Last but not least, *D. aruanus* is a small fish, and tagging experiments using very expensive chemicals such as ^{137}Ba can be managed from a financial point of view.

D. aruanus has a very limited home range like more than half of coral reef fish species in New Caledonia (Guillemot et al. 2011). Its reproductive strategy is broadly reflective of many families associated with coral reefs : its reproductive season extends over the whole warm season, and as many other reef fishes it changes sex and presents a protogynous hermaphrodism that is the normal pattern of reproductive ontogeny (Warner 1984). With a PLD of 3-4 weeks, *D. aruanus* spend approximately the same amount of time to disperse in the plankton than the majority of other teleost fish (Wellington and Victor 1989, Luiz et al. 2013). However, its PLD is longer than that of species usually used in connectivity studies (e.g., Planes et al. (2009), Saenz-Agudelo et al. (2012). *D. aruanus* larvae are also intermediate in terms of swimming capacities (Fisher 2005, Leis 2006).

All these criteria make *D. aruanus* potentially reflective of a broad range of demersal coral reef fishes, and a species particularly easy to survey for larval connectivity studies.

Chapitre 3

Wind-induced variability in larval retention in a coral reef system : A biophysical modelling study in the South-West Lagoon of New Caledonia

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Abstract

In the present work, a biophysical dispersal model is used to understand the role of the physical environment in determining reef fish larval dispersal patterns in the South-West Lagoon of New Caledonia. We focus on a reef fish species, the humbug damselfish *Dascyllus aruanus*, to investigate seasonal variability of simulated larval retention at the scale of a reef patch and at the scale of the lagoon, and to explore links between larval retention and wind variability. The model shows that retention exhibits considerable temporal variability and periodically reaches values much larger than anticipated. Non-zero larval settlement occurs over a large part of the lagoon. Nevertheless, settlement values decrease quickly away from the natal reef and mean dispersal distances are of order 25 - 35 km. Cross-correlation analyses indicate that weather conditions characterized by strong south east trade winds lead to low retention rates at both local (reef) and regional (lagoon) scales. By contrast, subtropical weather conditions characterized by weak winds result in high retention rates. These results suggest that large-scale weather regimes can be used as proxies for larval retention of the humbug damselfish in the South-West Lagoon of New Caledonia. Nevertheless, relatively small mean dispersal distances suggest that metapopulation dynamics occur on relatively small spatial scales.

Keywords : Biophysical model, Larval dispersal, Wind-driven transport, *Dascyllus aruanus*, Precompetency, Homing, New Caledonia

3.1 Introduction

The fragmentation of marine coastal habitats results in a geographical separation of local populations. Links among local populations are possible via the movement of individuals. When these connections are strong enough to have a measurable impact on local populations' growth rates, these populations constitute a metapopulation (Sale et al. 2006) and the exchanges between them are referred to as demographic connectivity (Cowen et al. 2007). Knowledge of demographic connectivity is required to understand metapopulation dynamics and the persistence and resilience of marine populations to anthropogenic pressures (Bernhardt and Leslie 2013), particularly in the context of implementing networks of marine protected areas (Sale et al. 2005). While progress has been made with older life stages, the larval dispersal component of connectivity has long been viewed as a black-box due to the many difficulties associated with directly observing a multitude of small individuals in a marine environment. In the past decade, advances in biophysical modelling (Miller 2007) and empirical techniques for connectivity assessment (e.g., genetic parentage analysis using DNA microsatellites and otolith transgenerational tagging) have permitted detailed investigation of early life dispersal (reviewed in Levin (2006), Cowen and Sponaugle (2009), Leis et al. (2011), Kool et al. (2013)). Although numerous marine species have pelagic larval durations that may last several weeks, Cowen et al. (2000) showed more than 10 years ago that the spatial scales of larval dispersal were not as large as anticipated (only 10 - 100 km *vs.* several hundreds of km as was previously thought). This result suggested that larval local retention (i.e., the ratio of the number of larvae that settled back to their natal population to the total number of larvae released there, Botsford et al. (2009)) could be important for the functioning and structure of marine populations. Since then, the idea of small scale demographic connectivity ensured by larval retention has been supported by modelling studies e.g., in the Great Barrier Reef in Australia (James et al. 2002), the Caribbean (Cowen et al. 2006, Cherubin et al. 2011), Hawaii (Christie et al. 2010) and the Indo-Pacific Ocean (Treml et al. 2012). Field observations also reported high levels of self-recruitment (i.e., the ratio of the number of larvae that settled back to their natal population to the total number of larvae that settled there, Botsford et al. (2009)) e.g., for reef fish species in Papua New Guinea (Almany et al. 2007, Planes et al. 2009, Saenz-Agudelo et al. 2012, Berumen et al. 2012), the Caribbean (Hogan et al. 2012) and the Great Barrier Reef (Harrison et al. 2012, Van der Meer et al. 2012).

The present challenge of larval dispersal research is to find out if this relatively "closed" population dynamics is the rule or the exception, and to understand its causes : does it result from local oceanography, larval life history traits, larval behavior, or a combination of these biotic and abiotic drivers. Local oceanography (currents, water

residence time) has been shown to be of great importance for explaining relatively closed population dynamics (Paris and Cowen 2004, Treml et al. 2012). Among life history traits, the length of the larval precompetency period, i.e., the period of time in which larvae may not settle (Jackson and Strathmann 1981), has been shown to be a key driver for local retention within the natal population (Black and Moran 1991, Paris and Cowen 2004, Treml et al. 2012). Whereas the precompetency period of reef-building coral larvae is relatively short (between 2 and 5 days, Heyward and Negri (2010)), it can reach several weeks for other reef species (Staaterman et al. 2012, Butler et al. 2011, Soria et al. 2012). Concerning larval behavior, several studies demonstrate that fish larvae have sensory capabilities coupled with strong swimming capabilities (Leis 2010) that facilitate local retention through homing. For instance, larvae are capable of olfactory discrimination and prefer the odor of their home reef (Gerlach et al. 2007). Acoustic (e.g., Radford et al. (2011)) and sun compass mechanisms (Mouritsen et al. 2013) have also been suggested to allow pelagic larvae to locate their natal reef. Modelling studies show that early active larval movement associated with orientation behavior is a mechanism for self-recruitment (James et al. 2002, Paris et al. 2005, Staaterman et al. 2012).

In this study we investigate the roles of local meteo-oceanography, precompetency period and homing behavior in determining reef fish larval retention in the South-West Lagoon of New Caledonia (SWL). The oceanography of the SWL has been particularly well studied over the last 40 years through *in situ* measurements and numerical models that provide insights on sediment transport and biogeochemical dynamics in the lagoon (Jarrige et al. 1975, Douillet 1998, Faure et al. 2010, Ouillon et al. 2010, Fuchs et al. 2012). Previous studies using Lagrangian tracers show that the SWL is well mixed by the joined action of tide currents, winds and swell which results in a rather low average water residence time (defined as the time needed for a water particle to leave the lagoon) of 11 days (Jouon et al. 2006, Ouillon et al. 2010). If we assume, as a first approximation, that larvae of marine species are passive entities during their precompetency period, and are therefore advected like water parcels, the short water residence time found in the SWL has enormous consequences for larval retention : species with a larval precompetency period longer than 11 days will be mostly flushed out of the lagoon.

In this study we focus on a coral reef damselfish, *Dascyllus aruanus*, a species that has precisely a precompetency period of $\sim$ 11 days (see section 3.2.3). We use a three-dimensional biophysical model to investigate larval retention of *D. aruanus* and its seasonal variability inside the SWL. We first describe the study species and region focusing on the oceanographic context. The biophysical model is then described and used to assess larval retention at two different spatial scales : SWL scale (“natal lagoon

retention") and local patch reef scale ("natal reef retention"). We define (1) "natal lagoon retention" and (2) "natal reef retention" as the ratio of the number of larvae released at the natal reef that settled (1) on any of the settlement reefs including the natal reef or (2) only on the natal reef, to the total number of larvae released at the natal reef. Larval retention was simulated under two opposite hypotheses regarding homing behavior : a strict-homing hypothesis and a no-homing hypothesis. Finally, to stress out the role of the local meteo-oceanography on larval retention, our results are linked to the synoptic-scale variability of the low-level circulation in New Caledonia that we can describe through the so-called weather regimes (Lefèvre et al. 2010). A sensitivity study is conducted using a longer (3 weeks) precompetency period and different larval release depths.

3.2 Material and methods

3.2.1 Study area

New Caledonia (19 - 23°S, 163 - 168°E) is an island located in the South West Tropical Pacific 1500 km east of Australia. The New Caledonia lagoon is surrounded by a barrier reef of exceptional size (1600 km in length, the second longest double barrier reef in the world, after the Great Barrier Reef) and is listed as a UNESCO World Heritage Site since 2008. The work presented here focuses on the South-West Lagoon of New Caledonia (SWL) which surrounds Nouméa, the island's main city (fig. 3.1). A network of 13 Marine Protected Areas (MPAs) has been established in this area to mitigate the increasing anthropogenic pressure on the lagoon. The SWL covers an area of about 2000 km^2 delimited by the coast on the eastern side and the barrier reef on the western side, extending from the Mato pass in the south to the Ouaraï pass in the north. Depth averages 20 m and varies from less than 1 m around islets to 60 m inside canyons. The lagoon ranges in width from 5 km (northern limit) to 40 km (southern limit) with a length along the north-west/south-east axis of about 100 km, and is connected with the Pacific Ocean by several deep passes.

3.2.2 Local meteo-oceanography in summertime

The two main forces driving circulation in the SWL are tides and winds (Douillet 1998). Wind-induced current velocities are approximately one order of magnitude higher than velocities generated by tides (Ouillon et al. 2010). Austral summer (from October to March) is dominated by southeasterly trade winds blowing from 60° to 160° at speeds averaging 8 m s^{-1} (Pesin et al. 1995). Recently, Lefèvre et al. (2010) identified four weather regimes occurring in New Caledonia during austral summer through an

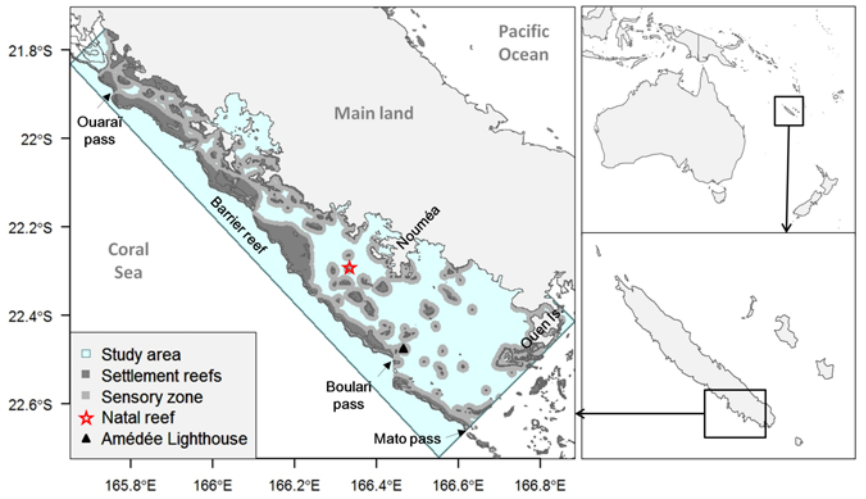

Figure 3.1 Study area : the South-West Lagoon of New Caledonia (SWL in light blue). Settlement reefs (in dark grey) are buffered by a 1-km sensory zone (in light grey).

objective classification applied to remote sensed winds for nine summer seasons from 1999 to 2008 (table 3.1). Three of these weather regimes (regimes 1, 3 and 4) exhibit low-level circulation dominated by SE trade winds. Regime 1 captures a strong, near steady and alongshore trade wind flow, averaging 8 m s^{-1} and is referred to here as "Strong SE Trade-wind". This regime is the most frequent, accounting for slightly less than a third of the austral summer days. Long spells of regime 1 are more favorable to the flushing of the lagoon by driving a general north-west drift. The SWL is thus mainly fed with oceanic waters at its southern end, through the different passes of the barrier reef as described in Jouon et al. (2006). This regime promotes the formation of upwelling events along the barrier reef (Marchesiello et al. 2010) and local wind acceleration in SWL due to flow splitting by the mountainous island (Lefèvre et al. 2010). The regional circulation in regime 4, called "Subtropical SE wind", also shows a near steady alongshore SE direction, but with weaker winds (5.2 m s^{-1} in average) associated with strong subtropical westerlies south of the island. Occurring for 27% of the days in Lefèvre et al. (2010) analysis, this regime is more frequent in early summer (November - December). Regime 3, called "Tropical SE wind", accounts for 22% of the days, occurs more frequently during the warm and wet period from January to March, and produces average winds of 5.2 m s^{-1}. The fourth regime, regime 2, called "Weak Easterly Circulation", exhibits a weak easterly airflow circulation (below 2 m s^{-1}) and the largest variability in wind direction. It is also the most transient and least robust regime, accounting for less than 8% of the days.

TABLE 3.1 Description of the four weather regimes defined in Lefèvre et al. (2010). Statistics of wind speed and direction were computed from remote sensed surface winds for the period 1999-2008 (adapted from Lefèvre et al. (2010)). SD = standard deviation.

	Name	% of occurence	Wind speed (m s^{-1})		Wind direction (°N)	
			Mean	SD	Mean	SD
Regime 1	Strong SE Trade-wind	30	8	2.2	122.5	21.6
Regime 2	Weak Easterly Circulation	7.4	1.4	2.3	120.6	94.8
Regime 3	Tropical SE wind	22.6	5.2	2.8	122.9	60.5
Regime 4	Subtropical SE wind	27.3	5.2	2.2	118.9	45.6

3.2.3 Study species

This study is part of a larger program focusing on the population connectivity of the Humbug damselfish (Pomacentridae), *Dascyllus aruanus*, in the SWL of New Caledonia. Here, we therefore use the larval life history traits of this species as the basis for our exploration of larval dispersal patterns in the SWL. *D. aruanus* is an obligate coral-dwelling Pomacentridae, found exclusively in lagoon habitats (Allen 1991), where it lives in well-mixed waters among branching coral colonies in spatially discrete groups of 2 - 80 individuals (Sale 1972a, Holbrook et al. 2000, Cole 2002). Coral colonies provide protection from predators and substrate for laying benthic eggs Coates (1980b), Mizushima et al. (2000). *D. aruanus* adults are sedentary benthic spawners that breed on a lunar cycle throughout the year (Pillai et al. 1985). Spawning peaks in summer, during which each female can spawn several times at 1 week (personal observation) to 2 months (Mizushima et al. 2000) intervals. Eggs remain in benthic nests for 3 days after which hatchlings are released into the plankton where they disperse on average 3 weeks (mean planktonic larval duration -PLD- Thresher et al. (1989), Juncker et al. (2007), Soeparno et al. (2012) prior to settling on adult reef habitats. Newly hatched larvae have well developed sensorial abilities (Leis 2010) and pomacentrids larvae are able to swim actively against currents during the second half of their pelagic larval phase (Fisher 2005). Furthermore, field evidence indicates that late-stage larvae of coral reef fish can detect the presence of a reef at a range of at least 1 km (Leis et al. 1996). Thus the sensorial and swimming abilities which allow the orientation of competent larvae to suitable recruitment habitat (Sweatman 1983, Holbrook et al. 2000) may be present in *D. aruanus* larvae as early as the age of 11 days (i.e., half of mean PLD).

3.2.4 Biophysical model

Larval dispersal was simulated with a biophysical model using version 3.0 of the Lagrangian tool Ichthyop (Lett et al. 2008). Ichthyop is a three-dimensional (3D) particle-tracking model designed to study the effects of physical and biological factors on the transport and settlement of ichthyoplankton. The biophysical model is based on an offline forcing of an individual-based model (IBM) by a 3D hydrodynamic model. The hydrodynamic model used here is the high-resolution 3D Model for Applications at Regional Scales (MARS3D, Lazure and Dumas (2008)). MARS3D provides 3D dynamic fields of current velocities which result from the combined actions of wind and tidal forcing. Several configurations of this model have been developed and validated in the SWL (Douillet 1998, Douillet et al. 2001, Ouillon et al. 2010). The configuration of MARS3D used in this study covers the SWL from 22.06° to 22.52°S and from 165.98° to 166.79°E. The model grid has a horizontal resolution of 500 m and 30 terrain following generalized sigma levels in the vertical dimension. This configuration is forced by realistic hourly winds at 4 km resolution obtained from the mesoscale Weather Research and Forecasting (WRF) model (Lefèvre et al. 2010). Simulated surface wind speeds and directions are very close to observations (Indice of Agreement IOA of 0.9 and RMSE $<$ standard deviation of observations for both speed and direction, section 3.6, table 3.2). Realistic wind forcing corresponds to the years 2003-2004. This period is neutral regarding ENSO phases. Tides are included in MARS3D through a lateral forcing using the Oregon State University TPXO.6 tides solution (Egbert et al. 1994) for 8 tidal constituents. The TPXO tides solution is refined further within ADCIRC (ADvanced CIRCulation model, Luettich et al. (1992)), by using an unstructured and very fine resolution mesh (from 500 to 25 m in the SWL, Lefèvre, pers. comm.).

Our larval transport IBM uses MARS3D model results covering the reproductive period of *D. aruanus* in New Caledonia i.e., from mid - September to late March. Outputs of MARS3D simulations were stored every 12 min as this time step is sufficient to account for the effects of tides. In the IBM, larvae are characterized by their latitude (°S), longitude (°E) and depth (m). Locations of individuals are updated every 5 min in three dimensions using the velocity fields stored from MARS3D interpolated in space and time via a forward-Euler integration scheme.

3.2.5 Simulations

In order to study the effect of wind regime on larval retention, we examined dispersal from a $\sim$ 500 m diameter patch reef located at the center of the SWL (called natal reef hereafter) where water residence time is close to the average of 11 days

(Ouillon et al. 2010). Location and extent of the natal reef and settlement areas in the SWL were defined as polygons based on GIS habitat maps provided by the atlas of coral reefs in New Caledonia (Andréfouët and Torres-Pulliza 2004). Since *D. aruanus* is ubiquitous in the SWL all reefs shallower than 20 m were considered as potential settlement habitats in the model. Hatching events (representing a release of 500 virtual larvae each) were simulated over the natal reef every 3 h over austral summer from mid-September 2003 to late March 2004 (i.e., 1320 simulations). For each release on the natal reef, larvae were randomly distributed throughout the water column from 0 to 20 m depth. Larvae from each hatching event were followed for up to 30 days (the largest value of PLD reported for *D. aruanus* (Soeparno et al. 2012)). We considered that larvae were initially transported passively by ocean currents during an 11-day precompetency period and that they became active afterwards with sensory and swimming capabilities that allowed them to detect and approach a settlement area. To do so, a non-explicit swimming behavior during the competency period was included by assuming that larvae could actively settle once at a given distance from a settlement reef. This distance was defined as 1 km (Leis et al. 1996). Any virtual larva located less than 1 km away from a settlement reef at any time between the end of the precompetency period and 30 days was then considered to have successfully settled.

Simulations were run under two alternative hypotheses about natal homing. Under the first hypothesis, settlement was supposed to be driven by strict natal homing : settlement was only allowed on the natal reef, i.e., competent larvae were only able to settle on the natal reef. We will refer to this hypothesis as the "strict homing hypothesis". Under the second hypothesis, settlement of competent larvae was allowed on the natal reef and in any other part of the SWL where suitable habitat for *D. aruanus* was available. We will refer to this hypothesis as the "no-homing hypothesis". A longer precompetency period of 3 weeks (21 days) was also tested. A sensitivity analysis to the larval release depth was also conducted using simulations run for a precompetency period of 11 days for both hypotheses regarding natal homing. As larvae are characterized by their latitude (°S), longitude (°E) and depth (m) at each time step, it is possible to know the release depth of each recruited larvae at the end of the simulation. Five release depth intervals were tested from 0 to 20 m. All post-processing computations were done using R-2.15.0.

3.2.6 Lagoon *vs.* reef retention

Simulation outputs were used to calculate larval retention at two different spatial scales. Larval retention was first computed for each simulation at the SWL scale. This retention, hereafter called "natal lagoon retention" (NLR), is defined as the ratio of the number of larvae released at the natal reef that settled on any of the settlement

reefs to the total number of larvae released at the natal reef. Larval retention was also computed for each hatching event at the natal reef scale. This local retention, hereafter referred to as "natal reef retention" (NRR), is defined as the ratio of the number of larvae released at the natal reef that settled back to that site to the total number of larvae released at the natal reef. Three retention time series extending from mid-September 2003 to early March 2004 were obtained : NLR under the no-homing hypothesis ; NRR under the no-homing hypothesis ; NRR under the strict-homing hypothesis. Note that under the strict-homing hypothesis NLR equals NRR.

3.2.7 Cross-correlations

To study the link between wind conditions and simulated *D. aruanus* larval retention, we calculated cross-correlations between wind and retention time series with a maximum lag of 30 days. We extracted hourly meridional and zonal wind components from the WRF model at the closest grid point to the natal reef and converted them into an along-shelf (V-component) and cross-shelf (U-component) coordinate system, rotated 60° anti-clockwise from true north, with V positive towards the north-west (300°) and U positive onshore towards 30°. Time series of daily probability of occurrence of the four weather regimes defined by Lefèvre et al. (2010) were also used in a cross-correlation analysis with simulated retention time series. We used the Spearman rank-order correlation coefficient (hereafter Spear-R) because the simulated retention values were not normally distributed. Given that autocorrelation of time series increases the risk to consider that correlations between series are significant when they are not, we accounted for autocorrelation of all time series explicitly in judging the significance of correlations by adjusting the degrees of freedom following Pyper and Peterman (1998) and Botsford and Paulsen (2000). Auto-correlation timescales of order 2-8 days, depending on the simulation and time series examined, were identified and the effective degrees of freedom were corrected accordingly. This correction reduced the effective degrees of freedom and consequently substantially increased the value of correlation required for a significant result. All reported correlation coefficients are significantly different from zero at the 95% confidence level.

3.2.8 Larval settlement maps

Larval settlement maps were plotted at the SWL scale from the simulations with the no-homing hypothesis. For each hatching event, the spatial distribution of settlers was computed for square grid cells of 0.01° spatial resolution. The proportional number of settlers (relative to the total number of released larvae = 500) in each grid cell was then averaged over all hatching events, as well as over two extreme decompositions of the simulation period : (1) hatching events whose precompetency period consisted of at

least 75% weather regime 1 (Strong SE Trade-wind, table 3.1) and (2) hatching events whose precompetency period consisted of at least 75% weather regime 4 (Subtropical SE wind, table 3.1). The centers of mass of settlement maps were then calculated as the weighted (by settlement) spatial average over grid cells. To enhance visibility, some results were converted to a logarithmic scale by calculating $\log_{10}(N_i + 1)$ where N_i is the total number of larvae, over all simulations, settling in grid cell i.

3.3 Results

3.3.1 Time series

The retention time series exhibit considerable temporal variability for both precompetency periods (fig. 3.2a and b). For an 11-day precompetency period, natal lagoon retention (NLR) ranges from 0% to 100% with a mean of 56.7% ($\pm$ 26.4% SD). Natal Reef Retention (NRR) ranges from 0% to 42.6% with a mean of 6.7% ($\pm$ 8.2% SD) under the strict-homing hypothesis. Under the no-homing hypothesis, NRR is considerably smaller, ranging from 0% to 23.0% with a mean of 1.4% ($\pm$ 2.3% SD). The three simulated retention time series are highly positively correlated (Spear-R 0.63, 0.82, and 0.73, for the NLR and NRR when no homing, NLR and NRR when strict homing, and NRR when no homing and NRR when strict homing time series, respectively). At both spatial scales (natal reef and natal lagoon) and under both hypotheses (strict and no homing), mean retention decreases as the precompetency period increases. For a 21-day precompetency period, NLR ranges from 0% to 86.4% with a mean of 32.9% ($\pm$ 20.9% SD). NRR ranges from 0% to 18.8% with a mean of 1.9% ($\pm$ 3.0% SD) under the strict-homing hypothesis. Under the no-homing hypothesis, it ranges from 0% to 4.0% with a mean of 0.3% ($\pm$ 0.5% SD).

Wind conditions simulated by WRF model over austral summer 2003-2004 show two predominant states (fig. 3.2c) representing each about a third of the time series. Periods corresponding to trade winds with wind speed > 8 m s^{-1} and steady direction (mean = 110°N $\pm$ 23 SD) alternate with periods with weak wind speed (< 5 m s^{-1}) and variable direction (mean = 160°N $\pm$ 88 SD). During trade wind episodes the along-shore wind component (V) is high towards North-West (mean = 8.6 m s^{-1} $\pm$ 2.0 SD) and the cross-shelf wind component (U) is oriented offshore (mean = -1.5 m s^{-1} $\pm$ 2.4 SD), whereas weak and variable wind episodes are characterized by a lower V (mean = 1.2 m s^{-1} $\pm$ 2.3 SD) and U component towards the coast (mean = 0.1 m s^{-1} $\pm$ 2.3 SD) (fig. 3.2c and d). Consistent with these conditions, wind regimes 1 (Strong SE Trade-wind) and 4 (Subtropical SE wind) are predominant during the study period (fig. 3.2e), occurring 35% and 43% of the study period, respectively. Regimes 2 (weak

easterly circulation) and 3 (tropical SE wind) winds represent 8% and 13% of the study period, respectively.

3.3.2 Cross-correlations

Natal lagoon retention (NLR)

For an 11-day precompetency period, the time series of NLR shows significant negative cross-correlations with the along-shore component of wind (fig. 3.3a) between days 2 and 9 after release, with an absolute maximum correlation coefficient of 0.3 occurring at a lag of 5 days. For a 21-day precompetency period, correlations are consistently negative at about -0.2 for lags of 3-20 days, albeit only marginally significant at lags of 12-13 days and 18-19 days (correlation coefficient of $\sim$ -0.3) (fig. 3.3b). Cross-correlations with the cross-shelf component are not significant. For both precompetency period lengths, NLR is negatively correlated with the probability of weather regime 1 (fig. 3.4a and b) between days 2 and 5 after release with absolute maximum correlation coefficients of 0.4. NLR is not significantly correlated to regime 4, but correlation coefficients are generally positive (fig. 3.4c and d) for both precompetency period lengths. Cross-correlations with regimes 2 and 3 are not significant.

Natal reef retention (NRR)

Time series of NRR under the strict-homing hypothesis show significant negative cross-correlations with the along-shore component of wind for both precompetency period lengths (fig. 3.3a and b). Significant correlations occur between days 3 and 10 and between days 5 and 13 after release for precompetency period lengths of 11 and 21 days respectively, with absolute maximum correlation coefficients of 0.3 for both precompetency period lengths (fig. 3.3a and b). Under the no-homing hypothesis NRR is not significantly correlated to along-shore winds for a precompetency of 11 days, but correlation coefficients are generally negative at the beginning of the precompetency period (fig. 3.3a). For a precompetency of 21 days, the time series of NRR under the no-homing hypothesis shows significant negative cross-correlations with along-shore winds between 5 and 7 days (fig. 3.3b), with an absolute maximum correlation coefficient of 0.2. Cross-correlations with cross-shelf winds are not significant.

For an 11-day precompetency period, NRR under the strict homing hypothesis is significantly and negatively correlated to regime 1 at day 4 with an absolute maximum correlation coefficient of 0.4 (fig. 3.4a) and NRR under the no-homing hypothesis is not significantly correlated to weather regime 1 at the beginning of the PLD, but the correlation coefficients are consistently negative (fig. 3.4a). For an 11-day precompetency

period, NRR is significantly and positively correlated with regime 1 between days 16 and 24 (no-homing hypothesis) and between days 17 and 19 (strict-homing hypothesis) with maximum correlation coefficients of 0.4 (fig. 3.4a). For a 21-day precompetency period with the no-homing hypothesis, NRR is significantly and negatively correlated with regime 1 between days 3 and 6, with an absolute maximum correlation coefficient of 0.4 (fig. 3.4b). For a 21-day precompetency period with the strict-homing hypothesis, NRR is significantly and positively correlated with regime 1 between days 21 and 24, with a maximum correlation coefficient of 0.4 (fig. 3.4b).

For an 11-day precompetency period, NRR under the strict-homing hypothesis is significantly and positively correlated to regime 4 between days 1 and 5 after release with a maximum correlation coefficient of 0.5 (fig. 3.4c). Under the no-homing hypothesis NRR is not significantly correlated to regime 4, but correlation coefficients are generally positive (fig. 3.4c). For a 21-day precompetency period and under both homing hypotheses, NRR is significantly and positively correlated with regime 4 between days 4 and 5 after release with maximum correlation coefficients of 0.5 (fig. 3.4d).
Cross-correlations with regimes 2 and 3 are not significant.

3.3.3 Settlement maps

For an 11-day precompetency period, the average settlement map over the 1320 hatching events covering austral summer has non-zero settlement in nearly every suitable habitat, north and south of the natal reef (fig. 3.5). Nevertheless, settlement is inhomogeneous over space, decreasing rapidly with distance from the natal reef and reaching very low values in the north and the south of the SWL (fig. 3.5). The mean dispersal distance is 25 km (35 km for a 21 day precompetency period) and the center of mass is located slightly north of the natal reef.

When hatching is followed by a precompetency period dominated at 75% by weather regime 1, the vast majority of larvae settled north of the natal reef (fig. 3.6a). By contrast, when the precompetency period is dominated at 75% by regime 4, larvae settle almost everywhere in the lagoon (fig. 3.6b). Settlement maps are very similar for a 21-day precompetency period (section 3.7, fig. 3.8).

3.3.4 Sensitivity to release depth

At both spatial scales (natal reef and natal lagoon) and under both hypotheses (strict and no homing), time series of retention corresponding to different depth of release are positively correlated. For instance, NLR of larvae released in the upper layer

(between 0 and 4 m depth) and NLR of larvae released in the bottom layer (between 16 and 20 m depth) are correlated with a Spear-R of 0.4. The extremely high variability in simulated larval retention is equally true for all release depth levels. Nevertheless time series show different magnitude depending on the depth of release. Larvae released in upper layers had significantly less chance to settle than larvae released in bottom layers (fig. 3.7). For example, NRR under the strict-homing hypothesis averaged 8.1% ($\pm$ 0.4 Standard Error) when larvae were released between 16 and 20 m depth, but only 4.8% ($\pm$ 0.2 Standard Error) when they were released between 0 and 4 m depth. These two values are significantly different (Student test p value <0.05).

3.4 Discussion

Results from our larval dispersal modelling study show that larval retention is highly temporally variable over a reproductive season in the South-West Lagoon of New Caledonia (SWL) at both lagoon and natal reef scales (fig. 3.2). Occasionally, we obtained much larger larval retention rates than suggested by short average water residence timescales reported by previous hydrodynamic studies in the SWL (Jouon et al. (2006), Ouillon et al. (2010), see section 3.1). These modelling studies assumed periodic tides and constant, uniform winds over the SWL corresponding to strong trade winds (SE, 8 m s^{-1}), the most frequent and long-lasting wind regime on New Caledonia (similar to Lefèvre et al. (2010) weather regime 1). Using realistic wind forcing, our results indicate that during periods of weaker (regime 4) winds, larvae can stay within the SWL for their entire precompetency period. Correspondingly, larval retention rates during these periods can be as high as 100% for natal lagoon retention (NLR), and 43%/23% for natal reef retention (NRR) with/without homing respectively, for a precompetency period of 11 days. High retention rates occurred occasionally even for larvae released in the upper part of the water column and/or having a longer precompetency period, although average retention levels were smaller than for larvae released at greater depth (fig. 3.7) and/or having a shorter precompetency period (fig. 3.2). Homing behavior significantly increased retention at the reef scale (fig. 3.2), but it is not strictly necessary and all model configurations and simulations had at least some time periods with high levels of local retention.

High retention rates do not imply that populations are "closed" at the scale of individual reefs. In our study, NRR and NLR are highly positively correlated (Spear-R 0.63 and 0.82, for NLR and NRR when no homing or strict homing, respectively), and non-zero settlement occurs over a large part of the SWL (fig. 3.5). Nevertheless, settlement rates are low far from the natal reef (fig. 3.5), suggesting that while there is non-zero exchange of genetic material over the entire SWL, population dynamics is dominated by processes occurring at smaller spatial scales. For example, protected

areas of order 10's of km in size should have significant local retention of larvae and therefore a non-negligible chance of achieving self-persistence (Burgess et al. 2014).

Natal reef retention (NRR) is generally called "local retention" (Botsford et al. 2009), i.e., the ratio of the number of larvae that settled back to their natal population to the total number of larvae released there. Local retention is clearly an important variable to consider in population dynamics studies. Indeed, an isolated population can persist if each individual produces enough offspring to replace itself in the next generation (Botsford et al. 2009, Burgess et al. 2014) and this number increases with local retention. However, local retention is in practice difficult to measure as the reproductive output (number of larvae released) of a particular population must be assessed. This problem can be partially avoided by measuring local retention as defined by Hogan et al. (2012), i.e., the ratio of the number of larvae that settled back to their natal population to the total number of larvae released there that settled anywhere. This variable has been estimated in the field by Hogan et al. (2012) for a damselfish in the Caribbean, a species with life history traits very similar to ours. They found a mean value of 21% ($\pm$12% SD) on the 7 sites sampled over a 3-years study period. Hogan et al. (2012) definition of local retention corresponds exactly to NRR divided by NLR in our study. Values of NRR/NLR we obtain in our simulations are 2.0% ($\pm$2.8 SD) for a precompetency period of 11 days and 0.8% ($\pm$1.0 SD) for a precompetency period of 21 days, i.e., respectively ten and twenty times smaller than the values assessed by Hogan et al. (2012). These differences are potentially due to differences in the sizes of natal *vs.* all settlement reefs. In our case, the natal reef represents only 0.01% of all settlement reefs in the SWL (fig. 3.1), whereas if the 7 sites considered by Hogan et al. (2012) have similar sizes, each site represents $\sim 1/7 = 14\%$ of the total habitat considered. In both our study and Hogan et al. (2012), the fraction of larvae retained locally is superior to the fraction of habitat that the focal site represents, indicating that local retention is superior to what would be expected for a uniformly mixed larval pool, despite numerical retention rates being quite different between the two studies. This highlights the importance of taking habitat area into account when assessing local retention rates.

In order to understand the extremely high variability in simulated retention at both lagoon and reef scales, we calculated cross-correlations of retention time series with wind and weather regime time series. We found significant negative cross-correlations between all simulated retention time series and along-shore winds (approximately winds from the south-east) during the precompetency period (fig. 3.3), except for NRR under the no-homing hypothesis with an 11-day precompetency period. These results show that larval retention variability in the SWL is partly explained by wind forcing. Strong along-shore winds represent unfavorable conditions for larval retention in

the SWL because resulting ocean circulation flushes larvae out of the lagoon before they have time to settle (Jouon et al. 2006, Ouillon et al. 2010). This overall pattern is reflected in the relationship between settlement and the wind regimes identified in Lefèvre et al. (2010). The strong SE trade wind regime (regime 1) is unfavorable to larval retention during the larval precompetency period (fig. 3.4a and b). By contrast, the subtropical regime (regime 4) is positively correlated to retention values during the first days of pelagic larval life for both precompetency period lengths studied (fig. 3.4c and d). This regime is characterized by weaker SE winds with more variable direction than regime 1, including the majority of cross-shelf winds observed during our study period. Both these features reduce mean wind forcing and increase retention. Surprisingly, regime 1 had a slight but significant favorable effect on natal reef retention if occurring during the competency period (at a lag of approximately 3 weeks whatever the precompetency stage duration, fig. 3.4a and b). This suggests that optimal settlement conditions result from a combination of weak, regime-4 winds during the early part of the precompetency period, followed by strong, regime-1 winds close to the time of settlement (e.g., January 15^{th} - 21^{st} 2004 ; fig. 3.2a and e). When larvae disperse towards the south of the lagoon during the precompetency period as a result of regime-4 winds, regime-1 winds are needed for these to return to their natal reef (located in the center of the study area). This relationship breaks down when natal lagoon retention is considered because in this case larvae can settle in many different places within the SWL and therefore do not have to be transported back to the central natal site.

As shown in Lefèvre et al. (2010), the low-level circulation in New Caledonia varies significantly in connection with large scale forcing mechanisms, such as the El Niño/Southern Oscillation (ENSO) or the Madden Julian Oscillation (MJO). For example, Lefèvre et al. (2010) show that high regime 1 winds are more prevalent during El Niño events, the opposite being true during La Niña events. Since dynamics and residence time in shallow lagoons are tied to low-level winds, this implies that the large scale forcing may modulate larval retention in a similar way. Our results therefore suggest that El Niño (La Niña) years will lead to lower (higher) larval retention than average years. Given the strong correlation between retention rates and wind conditions, weather regimes and their connection with large scale oscillations (ENSO, MJO) may be a useful predictor of settlement success. To confirm the applicability of these proxies for settlement in the entire SWL, we compared retention time series from the focal reef with those from four other reefs located throughout the SWL, and found significant quantitative agreement in all but the most coastal reef (section 3.8), suggesting that weather regimes are likely a good predictor of settlement at intraseasonal and interannual scales over the majority of the SWL.

Real larval dispersal and settlement is considerably more complex than what is represented in our model, and we have consciously chosen not to explicitly represent a number of processes, such as larval mortality and vertical migration, because very little is known about them in general and particularly for our study species. Other modelling studies have demonstrated potentially significant effects of such processes on larval dispersal and connectivity (Paris et al. 2007, Brochier et al. 2008, Yannicelli et al. 2012). At this stage, our modelling work focused on investigating the effect of meteo-oceanography on larval retention and we believe that most of our qualitative conclusions on favorable and unfavorable wind conditions for retention remain largely unchanged if larval mortality or vertical migration were added in the model. Supporting this belief, including homing in our model did not lead to significant qualitative changes in our results (figs. 3.3 and 3.4), though absolute retention rates were affected (fig. 3.2).

3.5 Acknowledgments

This work was supported by the COMPO project (Connectivity Of Marine POpulations, www.compo.ird.fr) through a grant from the French National Research Agency (ANR), grant n° 2010 JCJC 1701 01.

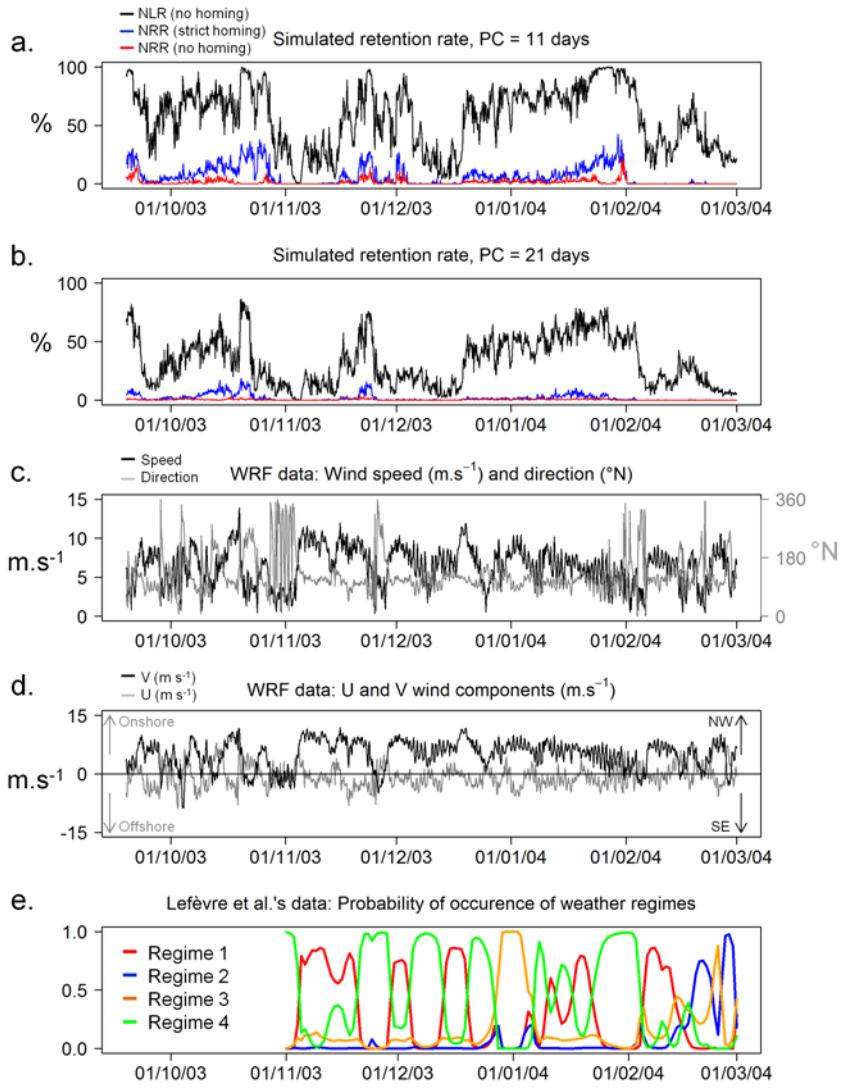

Figure 3.2 (a and b) Simulated retention rate time series : natal lagoon retention (NLR) under the no-homing hypothesis (black), natal reef retention (NRR) under the no-homing hypothesis (red) and NRR under the strict-homing hypothesis (blue) for two values of precompetency period (PC) : (a) PC = 11 days ; (b) PC = 21 days. Each time series provides larval retention rate corresponding to 1320 hatching events from mid-September 2003 to early March 2004 : retention rates are plotted for each corresponding release date. (c) WRF wind speed and direction ; (d) WRF U and V wind components ; (e) probability of occurrence of weather regimes defined by Lefèvre et al. (2010) from early November 2003 to early March 2004.

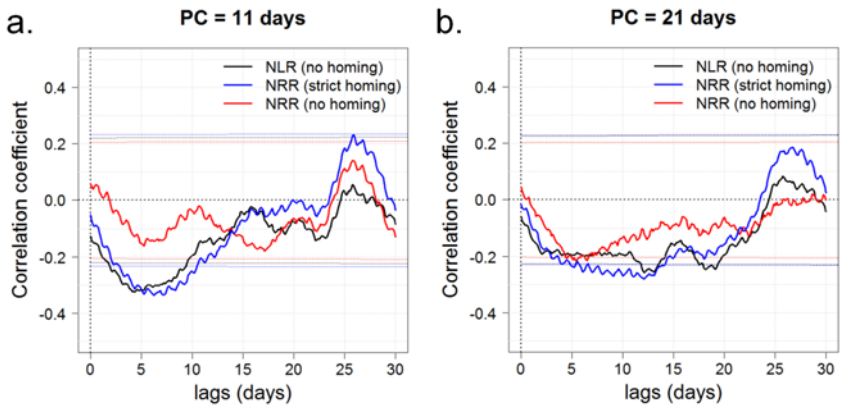

FIGURE 3.3 Cross-correlations between retention time series and along-shore wind component (V) derived from WRF model for two precompetency periods (PC) and under the hypotheses of strict or no homing. NLR : natal lagoon retention ; NRR : natal reef retention. Dotted lines represent cross-correlation critical values with a 95% level of confidence, adjusted to take into account temporal autocorrelation of time series.

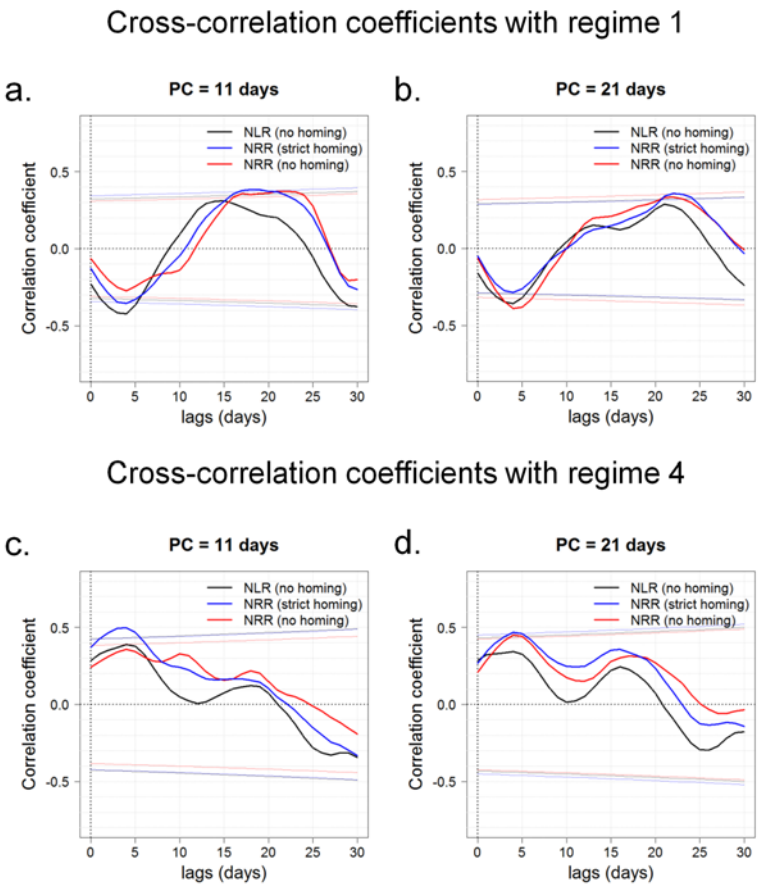

Figure 3.4 Cross-correlations between retention time series and weather regimes 1 and 4 for two precompetency periods (PC) and under the hypotheses of strict or no homing. NLR : natal lagoon retention ; NRR : natal reef retention. Dotted lines represent cross-correlation critical values with a 95% level of confidence, adjusted to take into account temporal autocorrelation of time series.

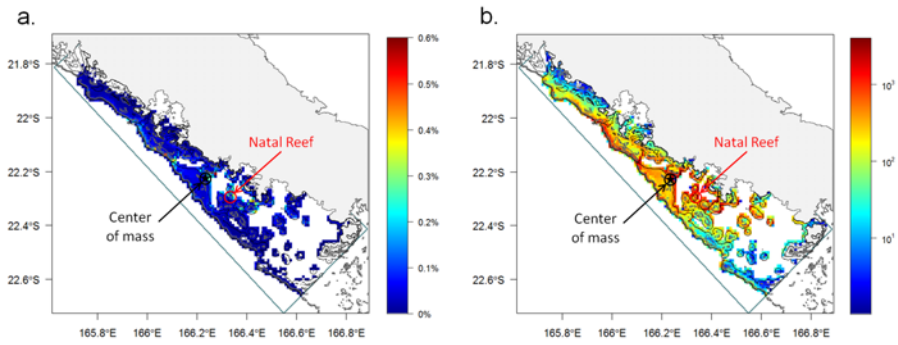

FIGURE 3.5 Maps of settlement using an 11-day precompetency period. For each simulation, the number of settlers was computed in square grid cells of 0.01° in size. For each grid cell, the number of settlers was (a) averaged over the whole simulated period (1320 simulations) and presented as a percentage of the total number of larvae released and (b) calculated on a logarithmic scale as $\log_{10}(N_i + 1)$ where N_i is the number of larvae settling in grid cell i.

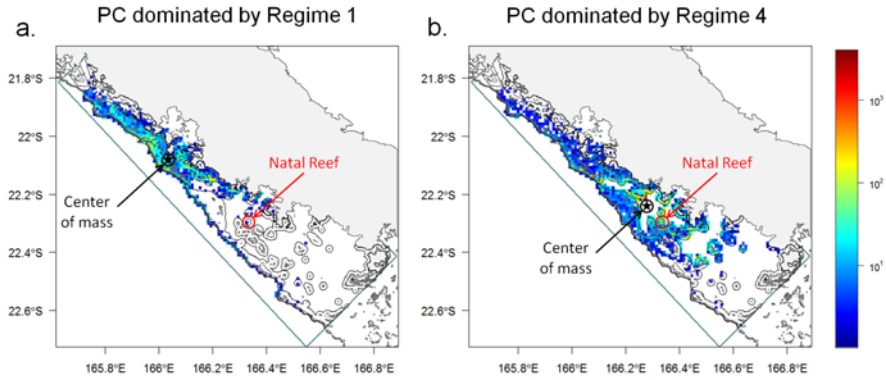

FIGURE 3.6 Maps of settlement using an 11-day precompetency period. The number of settlers was computed in square grid cells of 0.01° in size for simulations corresponding to hatching events followed by (a) a precompetency period dominated at 75% by weather regime 1 (42 simulations) and (b) a precompetency period dominated at 75% by weather regime 4 (34 simulations). For each grid cell, the number of settlers was calculated on a logarithmic scale as $\log_{10}(N_i + 1)$ where N_i is the number of larvae settling in grid cell i.

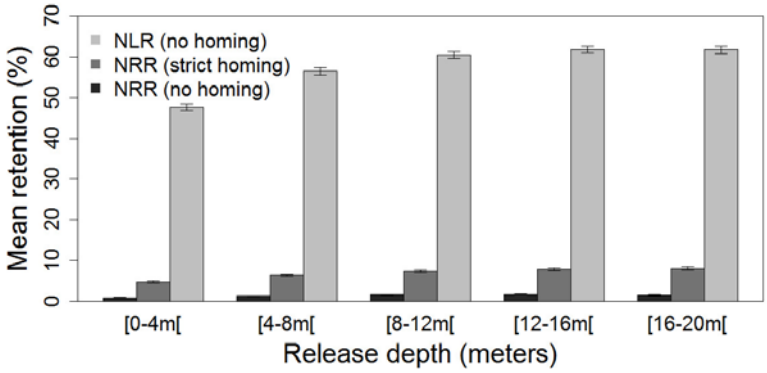

Figure 3.7 Mean retention over the austral summer 2003-2004 at natal reef scale (NRR) under no-homing or strict-homing hypotheses, and at lagoon scale (NLR) under no-homing hypothesis for different release depths for an 11-day precompetency period. Error bars represent standard errors.

3.6 Appendix A

Wind conditions predicted by the WRF model during austral summer 2003-2004 were compared to *in situ* data obtained from Amédée Lighthouse Météo-France weather station. This station is located along the barrier reef on a patch of reef close to the Boulari pass approximately 20 km offshore in front of Nouméa (fig. 3.1) and is representative of marine weather conditions in the SWL (Lefèvre et al. 2010). The station provides hourly wind speed (m s^{-1}) and direction (°N) with an accuracy of 1 m s^{-1} for wind speed and 10° for direction. The WRF predicted wind variables are extracted at the closest grid point to Amédée Lighthouse station and 6-hourly averages are computed for wind vector of both model and observations. Observations marked with low wind data (speed < 3 m s^{-1}) are removed prior to the statistical computation of wind direction. Validation of predicted wind with observation was done through statistical performance measures including the root mean square error (RMSE) and index of agreement (IOA) according to Lefèvre et al. (2010). The IOA is a measure of model skills in predicting variations about the observed mean ; a value above 0.5 is considered to be good, 1 means a perfect match (see Lefèvre et al. (2010) for more details about this index) (see table 3.2).

TABLE 3.2 Statistical comparison between WRF model predicted values and observations at Amédée Lighthouse weather station. SD = standard deviation.

	Wind speed (m s^{-1})	Wind direction (°N)
Amédée Lighthouse observations		
(Number of data = 3908)		
Mean	7.1	118.5
SD	2.9	60.3
Model - observations difference		
Bias	-0.8	9.4
RMSE	1.9	21.4
IOA	0.9	0.9

3.7 Appendix B

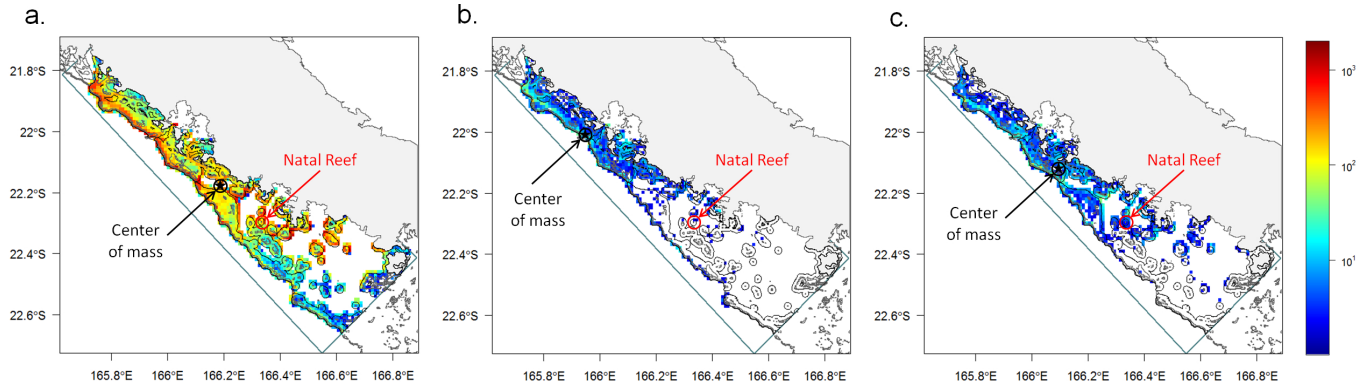

Figure 3.8 Maps of settlement using a 21-day precompetency period. For each simulation, the number of settlers was computed in square grid cells of 0.01° in size. For each grid cell, the number of settlers was calculated on a logarithmic scale as $\log_{10}(N_i + 1)$ where N_i is the number of larvae settling in grid cell i over (a) the whole simulated period (1320 simulations) and for simulations corresponding to hatching events followed by (b) a precompetency period dominated at 75% by weather regime 1 (42 simulations) and by (c) a precompetency period dominated at 75% by weather regime 4 (34 simulations).

3.8 Appendix C

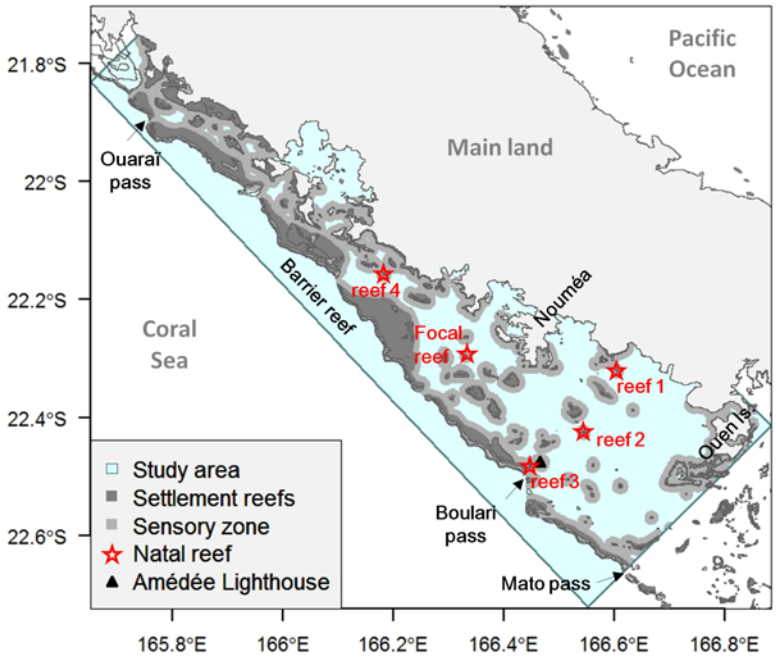

FIGURE 3.9 Location of the focal reef and the four reefs used to evaluate the impact of the focal reef choice on the results.

We based our study on larval releases from a patch reef located at the center of the SWL where water residence time is close to the average of 11 days (Ouillon et al. 2010). In order to evaluate the impact of this natal reef choice in the results, we simulated larval retention for 4 other natal reefs. We chose a reef located 25 km further south of the focal reef (reef 2) and a reef 20 km further north (reef 4) in areas where water residence time is also close to the average of 11 days (Ouillon et al. 2010), and 2 others reefs located on the barrier reef (reef 3) and close to the coast (reef 1) where water residence times are around 5 and 20 days respectively (Ouillon et al. 2010) (fig. 3.9). We simulated NRR under the stricthoming hypothesis and NLR under the no-homing hypothesis for an 11-day precompetency period and we calculated Spearman rank-order correlation coefficient (Spear-R) between the retention time series for the focal reef and the four other reefs. Average retention values ranged from 1.6% (± 2.1% SD, reef 3) to 7.3% (± 7.8% SD, reef 4) and from 48.9% (± 20.7% SD, reef 3) to 73.6% (± 18.1% SD, reef 2) for NRR and NLR, respectively. For NRR we found significant positive correlations of varying intensities between focal reef and

the reefs 1, 2, 3, and 4 (Spear-R 0.1, 0.2, 0.5 and 0.7 respectively). For NLR we found significant positive correlations between focal reef and reefs 3 and 4 (Spear-R 0.3 and 0.7), a not significant positive correlation between focal reef and reef 2 (Spear-R 0.1), but a significant negative correlation between focal reef and reef 1 (Spear-R -0.2). We relate the singularity of reef 1 to its position in a coastal area where gyres are generated by trade winds (Douillet et al. 2001). However in these gyres areas (North of Ouen Island in the southeast part of the SWL and in bays, fig. 3.9) the habitat of our study species is scarce. For these reasons we strongly believe that the conclusions of our study based on the focal reef remain meaningful for a large part of the SWL where our study species is present (patch reefs and barrier reefs).

Chapitre 4

Evaluation of transgenerational isotope labeling of embryonic otoliths in a coral reef damselfish with single and repeated injections of enriched ^{137}Barium

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Abstract

Quantifying the larval dispersal component of population connectivity is extremely challenging due to the many difficulties associated with directly observing larvae in their marine environment. Transgenerational isotope labeling is a recent empirical technique that addresses this challenge. It relies on the transmission of an artificially enriched stable isotope (e.g., ^{137}Ba) from gravid females to the embryonic otoliths of their offspring, allowing for mass permanent marking of larvae. Before implementing transgenerational isotope labeling in the wild, it is essential to investigate the transmission longevity of the mark from females to larvae and to assess the potential negative effects on females and their offspring. We injected females of the Humbug damselfish, *Dascyllus aruanus*, with an enriched ^{137}Ba solution and reared the resulting progeny to test the marking success and the transmission longevity of the mark, as well as determine potential effects of transgenerational isotope labeling on spawning frequency and size of 1-day eggs and 2-day larvae. Three different single-injection dosages (0.5, 1 and 5 µg of ^{137}Ba g^{-1} fish weight) were tested, as well as monthly repeated injections of the lowest dosage over a whole reproductive season. We implemented a new method that allows extracting otoliths of newly hatched larvae and analyzing them using laser ablation coupled plasma mass spectrometry (ICP-MS). We showed that for *D. aruanus*, injection with a low dose (0.5 µg of ^{137}Ba g^{-1} fish weight) produced consistently significantly marked larvae with a half-life for successful enriched Ba mark transmission of approximately 1 month, and that monthly repeated injections of this dose did not negatively impact spawning success or condition of eggs and larvae. Monthly repeated injections of enriched Ba isotope injections at 0.5 µg of ^{137}Ba g^{-1} fish weight will therefore present an effective means of mass marking *D. aruanus* larvae throughout an entire reproductive season.

Keywords : Barium isotopes, Connectivity, *Dascyllus aruanus*, LA-ICP-MS, Otolith microchemistry, Transgenerational marking

4.1 Introduction

Knowledge of marine metapopulation dynamics is essential to better understand population persistence and effectively manage and conserve marine resources and biodiversity. For this reason, quantifying demographic connectivity, i.e., the exchange of individuals among discrete populations (Cowen et al. 2007), has been identified as a priority area for research (Hixon 2011, Sale et al. 2005). While progress has been achieved with older life stages, the larval dispersal component of connectivity has long been viewed as a black-box (Sale et al. 2010) and its quantification remains challenging due to the many difficulties associated with directly observing a multitude of small individuals subject to high mortality rates in a marine environment (Thorrold et al. 2001).

Several empirical techniques have been developed and successfully applied during the last decade to address this knowledge gap (Leis et al. 2011) : analyses of trace elements in calcified structures (Carson et al. 2010), genetic approaches focusing on the assignment of individuals to populations of origin (assignment methods) (Hogan et al. 2012) or to specific parents (parentage analyses) (Saenz-Agudelo et al. 2011, 2012), and artificial chemical marking of calcified structures (Almany et al. 2007, Jones et al. 2005). Analyses of trace elements in calcified structures or assignment methods are potentially of limited value at small spatial scales due to homogeneity of genetic and microchemical signatures. By contrast, parentage analyses and artificial chemical marking are not constrained by the lack of difference among populations because they rely on unequivocal markers that uniquely assign individuals to their populations of origin. Artificial chemical marking of calcified structures has been accomplished by immersing embryos in solutions of fluorescent compound (Jones et al. 1999, 2005) or larvae in solutions of enriched stable isotope solutions (Munro et al. 2008, Woodcock et al. 2011). The major limitation of applying an immersion method in the wild is that it requires collecting and handling eggs.

An alternative artificial chemical marking method, called transgenerational isotope labeling, overcomes this limitation by marking individuals *in situ* before birth (Thorrold et al. 2006). This method relies on the transmission of an enriched stable isotope (e.g., ^{137}Ba) from gravid females to the embryonic otoliths of their offspring. The injection of a single female is sufficient to mark thousands of larvae that female will produce within the weeks following injection. Otoliths are then collected from-larvae or juveniles, and marked individuals are identified by measuring Barium (Ba) isotope ratios in otolith cores using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Thorrold et al. (2006) successfully applied transgenerational isotope labeling to both a benthic and a pelagic spawning species (respectively

a clownfish, *Amphiprion melanopus*, and black sea bass, *Centropristis striata*). Since then, transgenerational marking using enriched Ba isotopes has been successfully applied in the field to another clownfish (*A. percula*) and a butterflyfish (*Chaetodon vagabundus*) (Almany et al. 2007). This method has also been validated experimentally in captivity for 2 marine fishes (a clownfish, *A. percula*, Roy (2008) ; and a coral reef grouper, *Epinephelus fuscoguttatus*, Williamson et al. (2009)), 2 anadromous fishes (brown trout, *Salmo trutta*, Huelga-Suarez et al. (2011) ; and Atlantic salmon, *S. salar*, Huelga-Suarez et al. (2012)) and 3 freshwater fishes (golden perch, *Macquaria ambigua*, Munro et al. (2009) ; a goby, *Mogurnda adspersa*, Starrs et al. (2014) ; and a rainbowfish, *Melanotaenia splendid*, Starrs et al. (2013)). Pecl et al. (2010) applied this technique to several cephalopods (*Sepioteuthis australis*, *Euprymna tasmanica*, *Octopus pallidus*, and *O. maorum*) which have statoliths. Transgenerational marking techniques have also been developed using enriched isotopes of Strontium (Starrs et al. 2014, 2013, Zitek et al. 2013) or simple elemental Strontium (Buckley et al. 2007, Hobbs et al. 2012, Kuroki et al. 2010, Shippentower et al. 2011). Barium and Strontium have approximately the same ionic radius as Calcium which makes possible their deposition in the calcified matrix of otoliths (Campana 1999). The advantage of the stable isotope ^{137}Ba is that it does not present isobaric interferences with other ions, a crucial property for the ICP-MS analysis.

In addition to validating the effective maternal transmission of enriched stable isotopes to embryonic otoliths, it is also important to assess the transmission longevity, i.e., the time during which an injected female produces marked larvae. Transmission longevity is presumed to be species specific, relying on the elimination rate of the enriched stable isotope from the body of the female and may also depend on the injected Ba dose. Previous studies showed that maternal transmission longevity could range from 2 weeks for the cephalopod *Sepioteuthis australis* injected at 4.5 µg of ^{137}Ba g^{-1} body weight (Pecl et al. 2010) to 6 months for the freshwater rainbowfish *Melanotaenia splendida* injected at 20 µg of ^{137}Ba g^{-1} fish weight (Starrs et al. 2013, 2014). When using transgenerational marking in the wild, it may be necessary to ensure that injected females produce marked larvae throughout the whole reproductive season, for example to assess the seasonal variability of larval connectivity. In this view, if the maternal transmission longevity of the species is shorter than the duration of the reproductive period, injections must be repeated. Whereas a few studies have explored the potential adverse effects of transgenerational isotope labeling on adult females (Roy et al. 2013, Williamson et al. 2009) and offspring (Munro et al. 2009, Starrs et al. 2013, 2014, Williamson et al. 2009, Roy 2008), to our knowledge the efficiency and side effects of repeated injections of females have never been assessed. Furthermore, as transgenerational marking with rare isotopes can be quite expensive, it is important to test a range of dosage levels to identify the minimum effective dose.

In this context, the objective of this study is to test the efficacy of transgenerational ^{137}Ba isotope labeling for the coral reef damselfish *Dascyllus aruanus* and to identify an optimal dose for this species taking into account potential side effects of single and repeated injections on spawning success, eggs and larvae size. Furthermore, we describe a new method to extract and analyze otoliths from newly hatched larvae.

4.2 Methods

4.2.1 Study species

The Humbug damselfish, *D. aruanus* (fig. 4.1a) is a protogynous hermaphrodite Pomacentridae (Coates 1982) found exclusively in lagoon habitats throughout the Indo-Pacific region (Allen 1991). This sedentary fish lives among branching coral in spatially discrete groups with up to 80 individuals but typically less than 10 (Holbrook et al. 2000). Sex ratio is female-biased for colonies of less than 10 individuals (single-male polygynous group) and change to unity as group size increases (Fricke 1977, Fricke and Holzberg 1974). Branching coral provide protection from predators and substrate for laying benthic eggs (Coates 1980b, Mizushima et al. 2000). Spawning peaks in summer (October to March in New Caledonia), during which time each female can spawn several times at one week (Fricke and Holzberg 1974) to 2 months (Mizushima et al. 2000) intervals. After spawning, males guard the eggs for 3 days until they hatch.

4.2.2 Fish sampling and handling

Twelve discrete colonies of *D. aruanus* were collected with their branching coral in the South-West Lagoon of New Caledonia (SWL) in November 2011. To collect a colony, two divers approached the coral so that the fish hid among the branches. The branching coral was then covered with a large plastic bag, detached from the substrate, carried up to the boat and placed in a seawater tub. The colonies were then transported to the lab and the biggest individuals ($>$ 45 mm total length) were selected so that each colony comprised 5 to 7 mature fish. Each colony was then transferred to an 80 l aquarium with its own branching coral. The twelve aquaria were connected to a flow-through seawater system and exposed to ambient temperatures (26 - 29 °C) and salinity. Fish were fed twice daily a diet consisting of frozen Artemia and a mix of cut frozen tuna, squid and mussel. A programmed light system provided a natural photoperiod to all aquaria. All experimental fish were subject to an acclimation period of 11 months before beginning experimental trials in October 2012.

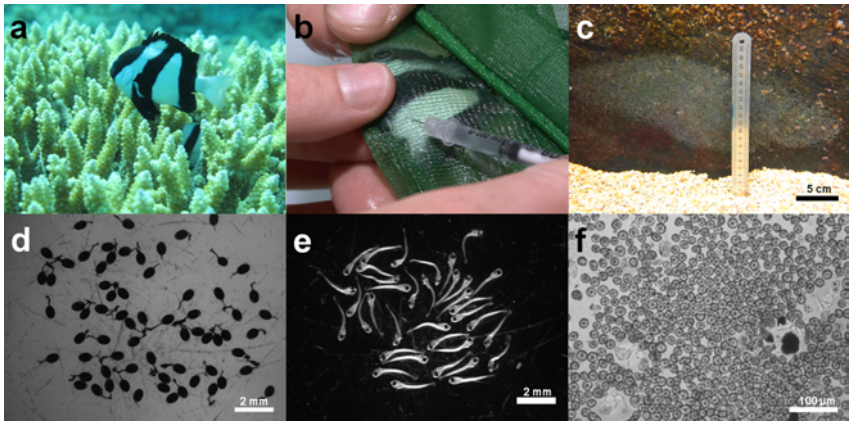

Figure 4.1 a) Adult *D. aruanus* on its branching coral colony, b) injection of an adult *D. aruanus* with enriched ^{137}Ba solution, c) clutch attached to a side of an aquarium, d) eggs sampled from a clutch and colored for measurement in the software program ImageJ, e) 2-day larvae sampled from a clutch, f) otoliths extracted from 2-day larvae originating from the same clutch ready for LA-ICP-MS analyses.

4.2.3 Enriched ^{137}Ba solution preparation

A Barium carbonate ($BaCO_3$) highly enriched in stable ^{137}Ba isotope (81.9%) and depleted in ^{138}Ba (17.4%) as compared to natural Ba isotope (11.32% ^{137}Ba and 71.66% ^{138}Ba), was purchased from Oak Ridge National Laboratory (Tennessee, USA). To obtain a solution in chloride form, 179.6 mg of $BaCO_3$ powder was dissolved in a small amount of ultrapure water with 1 ml of HCl (36%). Then, ultrapure water was added to obtain 25 ml of a $BaCl_2$ stock solution at 5 mg Ba ml^{-1}. The stock solution was then diluted to the desired concentrations with ultrapure water and neutralized with a solution of $NaHCO_3$ at 54 mg ml^{-1} (see section 5.6).

4.2.4 Enriched ^{137}Ba injections

Fish were anesthetized by adding 20 ml of alcoholic clove-oil solution (5%) in the aquarium, and captured using hand nets. All fish of a colony were transferred to a seawater tub. As *D. aruanus* does not present any sexual dimorphism, all fish of the colony were measured to the nearest mm total length and injected with the appropriate volume of ^{137}Ba solution (see section 5.6). Injections were performed with a 0.3 ml insulin syringe through the bodywall into the abdominal cavity (fig. 4.1b). Five different treatments were given to two replicate colonies each (table 4.1) : single injections of 0.5, 1 and 5 µg of ^{137}Ba g^{-1} fish weight (treatments T0.5, T1 and T5,

respectively) and monthly repeated injections of 0.5 µg of $^{137}Ba\ g^{-1}$ fish weight and 0.9% sodium chloride (NaCl) (treatments T0.5rep and TNaClrep, respectively). Injections of NaCl were used to distinguish between the effect of Barium accumulation over time and the potential effect of repeated manipulations (anesthesia and injection). Two control colonies were not manipulated at all. From the beginning of the spawning period in October 2012, we waited for at least 3 spawning events per colony before starting the injections. Injections began a few days before the full moon between mid-January 2013 and mid-February 2013 depending on the colony (table 4.1), after which time fish were monitored for a period of 4 months.

Table 4.1 Treatments used to evaluate mark success and effect of injections on spawning success and 1-day eggs and 2-day larvae size. FW = fish weight.

Treatment	Barium dosage (µg of ^{137}Ba g^{-1} FW)	Number of injection(s)	Date of injection(s)	Number of colonies	Total number of clutches collected to assess :		
					Mark success	Effects on 1-d eggs	Effects on 2-d larvae
Control	0	0	-	2	1	10	9
T0.5	0.5	1	11/01/2013	2	12	13	19
T1	1	1	13/02/2013	2	6	12	11
T5	5	1	29/01/2013	2	8	18	14
T0.5rep	0.5	4	11/01/2013	2	18	25	28
			13/02/2013				
			13/03/2013				
			12/04/2013				
TNaClrep	0	4	13/01/2013	2	-	23	19
			13/02/2013				
			13/03/2013				
			12/04/2013				
			Total	12	45	101	100

4.2.5 Spawning success and larval rearing

Spawning success was monitored daily in each colony over a period of 195 days from the first observed spawning event (the 25th of October 2012 in colonies 4 and 9) to the last one (the 7th of May 2013 in colony 1). Spawning events were generally synchronous among females of the same colony, though occasionally individuals were observed to spawn 1 - 2 days before or after the main spawning event. The periodicity of spawning in each colony was calculated as the ratio of the number of days between the first and the last spawning events observed in that colony to the number of spawning events observed in the same period. Females attached their eggs to the side glasses of aquaria (fig. 4.1c). Around 10% of each clutch was collected on the morning after spawning using a scraper and a pipette, and was stored in 80% Ethanol for 1-day egg measurements (fig. 4.1d). The remaining clutch was collected on the 3rd day after spawning and transferred to 2 l larval-rearing aquaria containing seawater having the same physicochemical characteristics as the parental aquarium. Hatching took place the night after transfer. Larvae were not fed until collection two days later (fig. 4.1e). Two-day larvae were stored in 80% Ethanol for otolith extraction and standard length (SL) measurements. As several females were present in each aquarium and spawning events were synchronous, it was not possible to separate the eggs produced by different females of the same colony. Thus, all eggs produced in an aquarium during one spawning event were pooled.

4.2.6 Otolith preparation and analysis

A total of 45 clutches, including a control clutch, were analyzed to assess marking success as a function of dosage and experimental protocol (table 4.1). Otoliths of 2-day larvae were extracted by dissolving organic tissue of larvae in commercial bleach (NaClO at 2.6%). Around 0.5 ml of larvae was put in a 1.5 ml Eppendorf Tubewith 1ml of bleach. The tube was shaken with a vortex and then centrifuged. The supernatant was removed and the operation (bleaching, shaking, centrifugating) was repeated twice more, so that the larvae were totally dissolved. After the third centrifugation the supernatant was removed and the operation repeated three times with ultrapure water, so that the otoliths were well rinsed. Then, the tube was shaken and poured in a glass Petri dish with the help of a wash bottle to ensure that all otoliths were removed from the tube. The Petri dish was placed under a binocular microscope and a clockwise rotary movement was applied until the otoliths gathered in the middle of the Petri dish. It is worthwhile noting that this does not work with a plastic Petri dish. Water in excess was removed using a pipette and then evaporated by placing the Petri dish above a hot plate to speed up the process. Aggregated otoliths were then collected all together using a piece of tape that was later mounted on a clean petrographic

slide with double-sided tape and then covered with a thin layer of cyanoacrylic glue to prevent them from being dislodged (fig. 4.1f). Each otolith aggregation sample obtained this way corresponded to one spawning event from one colony. Ba isotope ratios were measured in aggregated larval otoliths with a New Wave Research 213 nm UV laser ablation system connected to an Agilent 7500cs inductively coupled plasma mass spectrometer (ICPMS) located at Charles Darwin University (Darwin, Australia). Each analysis lasted 60 s including 10 s of background and 50 s of ablation (laser activated). A reference standard (NIST 612, National Institute of Standards and Technology) was analyzed at the beginning and end of each session to account for mass bias and machine drift throughout the analyses. Operating conditions of the laser and ICP-MS are provided in table 4.2. Between 3 and 5 ablations were randomly performed through each otolith aggregation sample so that around 10 otoliths were ablated per clutch. Each sample was analyzed for ^{43}Ca, ^{137}Ba and ^{138}Ba.

Table 4.2 Operating conditions of the New Wave Research 213 nm UV laser and Agilent 7500cs inductively coupled plasma mass spectrometer (ICP-MS) used for the otoliths analysis.

Laser parameters	
Wavelength	213 nm
Mode	Q-switched
Frequency	5 Hz
Laser power	90%
Beam density	9 - 11 J cm^{-2}
Carrier gas	Ar 1.01 l min^{-1}
Ablation mode	Spot
Beam diameter	55 µm
ICP-MS parameters	
Optional gas	He (140)
Dwell times	^{137}Ba & ^{138}Ba (300 ms) ; ^{43}Ca (30 ms)
Cone	Nickel sampler cone and nickel skimmer cone
Detection modes	Pulse and analog

4.2.7 Eggs and larvae measurements

One-day eggs

A total of 101 clutches were analyzed to assess the effect of injections on 1-day egg size (table 4.1). Measurements were performed using the plug-in ObjectJ (http://simon.bio.uva.nl/objectj/2-Tutorial.html) for the software program ImageJ (http://rsbweb.nih.gov/ij/, Schneider et al., 2012). Eggs were colored with Toluidine blue dye, separated using tweezers under a binocular microscope and photographed with a Scion CFW 1308C digital camera at X20 magnification. Images (fig. 4.1f) were calibrated in ObjectJ and the software automatically marked each egg with the

best fitting ellipse and recalculated the corresponding diameter for a perfect sphere with the same silhouette area (called "egg diameter" hereafter). A minimum of one hundred 1-day eggs were measured per clutch, except for 13 clutches for which only 46 to 87 eggs were measured. A total of 10,804 eggs were measured to the nearest µm.

Two-day larvae

A total of 100 clutches were analyzed to assess the effect of injections on 2-day larvae size (table 4.1). Photos of each sample of larvae (fig. 4.1e) were taken using the same microscope and camera than for eggs. Measurements of SL were performed using the segmented line tool of ImageJ. A minimum of one hundred 2-day larvae were measured per clutch, except for 15 clutches for which only 50 to 88 larvae were measured. A total of 9,771 larvae were measured to the nearest 0.01 mm.

4.2.8 Statistical analysis

All statistical analyses were conducted using the R-2.15.0 statistical environment. Mixed-effects models were computed with packages *nlme* (Pinheiro et al. 2011) and *MASS* (Venables and Ripley 2002). Percentages of variance explained by random factors were calculated by extracting standard deviations of random effects from models summaries, squaring them to get the variances, and then expressing them as percentages of total original variances (Pinheiro and Bates 2000). All statistical tests were considered significant at the 5% level.

Marking success

Isotope abundances measured in otoliths by LA-ICP-MS were first blank-subtracted. Ba ratios were then computed and corrected for mass bias and machine drift using the reference standard. The ratio $^{138}Ba/^{137}Ba$ in the reference standard was assumed to be the same as the relative natural abundance (i.e., 6.38) based on the International Union of Pure and Applied Chemistry values of isotopes abundance (Berglund and Wieser 2011). Following Munro et al. (2008), data were smoothed using a six-point running mean to decrease noise. Ba ratio measurements corresponding to ^{43}Ca values less than 1500 counts per second were excluded from analyses to remove measurements performed in the glue, leaving only those Ba ratios resulting from laser ablation of the otoliths, which are principally composed of $CaCO_3$. Mean Ba ratios from the treatment samples were then compared against ratios in the control sample using Mann-Whitney-Wilcoxon (MWW) tests to determine if mark transmission was successful. Mean Ba ratios (y), resulting from single injection treatments, were modeled as an exponential decay function of number of days post-injection (t) using a nonlinear mixed-effects

model fitted by maximizing the restricted log-likelihood with treatment (3 levels : T0.5, T1 and T5) as fixed effect and colony (numbered from 1 to 6) as random effect :

$$y = \bar{y_c} - \alpha \cdot \exp^{-\beta \cdot t} \tag{4.1}$$

with $\bar{y_c}$ the control mean Ba ratio.

The half-life ($t_{1/2}$) for successful enriched Ba mark transmission was calculated as follows :

$$t_{1/2} = \frac{\ln(2)}{\beta} \tag{4.2}$$

Effect on spawning success

To assess the potential effect of injection on spawning success, a generalized linear mixed model using penalized quasi-likelihood was fitted with a binomial distribution for daily spawning success (0 = no spawning event, 1 = spawning event). Treatment (6 levels : Control, T0.5, T1, T5, TNaClrep, T0.5rep) and time (days numbered from 1 to 195) were specified as fixed factors. The effect of colony (numbered from 1 to 12) was specified as random.

Effect on eggs and larvae

In order to detect potential effects of treatment on 1-day egg diameter and 2-day larval SL, we modeled these response variables using two mixed-effects models with polynomial terms fitted by maximizing the restricted log-likelihood. Treatment (6 levels : Control, T0.5, T1, T5, TNaClrep, T0.5rep) was specified as a fixed linear factor. The effect of time (number of days elapsed from the beginning of the experiment to the date that the clutch is produced) was also specified as fixed and modeled with a cubic function to represent temporal variability observed in the response variables. The effect of colony (numbered from 1 to 12) was specified as a random linear effect.

4.3 Results

4.3.1 Validation of Barium isotope markers

The mean Ba isotope ratio in otoliths of control larvae was 6.2 ($\pm$ 0.1 standard error (SE)). For all treatments, Ba isotope ratios in larval otoliths of the first spawned clutch differed considerably from the ratio in otoliths of control larvae (MWW tests p.values < 0.0005) even if variability among clutches within treatment was strong, ranging

from 1.0 (± 0.1 SE) in colony 7 to 4.5 (± 0.2 SE) in colony 8, both at a dose of 5 µg of ^{137}Ba g^{-1} fish weight (figs. 4.2 and 4.3). For all single injection treatments (T0.5, T1 and T5), Ba isotope ratios increased with time after injection (fig. 4.2). The Ba ratio in larval otoliths was still significantly different from the control until 108, 59 and 98 days post-injection for treatments T0.5, T1 and T5, respectively (fig. 4.2, MWW tests p.value < 0.005 ; 0.05 and 0.0005 respectively), with a non-significant Ba ratio for a clutch spawned by colony 7 (T5), 82 days post-injection (MWW tests p.value > 0.05). The nonlinear mixed-effects model showed no significant differences in mark transmission success among the three single injection treatments (p.values > 0.05, table 4.3a). The random factor colony explained a very small part of variation in Ba ratios (table 4.3a). Without treatment and colony effects, the estimated parameters of the exponential decay, α and β, were equal to 4.5316 and 0.0218 respectively (table 4.3b). The half-life for successful enriched Ba mark transmission was 32 days (fig. 4.2). For the repeated injection treatment (T0.5rep), the Ba isotope ratios consistently decreased immediately after an injection, and then tended to increase over time between injections (fig. 4.3). Non-significant Ba ratios were measured for colony 12 for two clutches spawned 16 and 30 days post-1st injection (fig. 4.3b, MWW tests p.value > 0.05). All clutches were successfully and consistently marked after the second injection (fig. 4.3, MWW tests p.value < 0.0005).

4.3.2 Spawning success

The generalized linear mixed model showed no significant effects of time and treatments on spawning success (p.values > 0.05, table 4.4). The random factor colony explained a very small part of variation in spawning success (table 4.4). The average periodicity of spawning of the 12 colonies was 10.7 days (± 2.4 standard deviation (SD)) (table 4.5).

4.3.3 Size of 1-day eggs and 2-day larvae

In the two control colonies, the average diameter of 1-day eggs over the whole time of experiment was 597 µm (± 30 SD) and the average SL of 2-day larvae was 2.54 mm (± 0.12 SD). For the ten treated colonies, the average size of eggs and larvae were variable among and within treatments. The average diameter of 1-day eggs ranged from 589 µm (± 33 SD ; colony 11, treatment T0.5rep) to 607 µm (± 30 SD ; colony9, treatment TNaClrep). The average SL of 2-day larvae ranged from 2.47 mm (± 0.11 SD ; colony 7, treatment T5) to 2.54 mm (± 0.15 SD ; colony 10, treatment TNaClrep). The response variables (1-day egg diameter and 2-day larval SL) varied during the course of the experiment for all levels of treatment and between colonies within the same treatment (fig. 4.4). The mixed-effects model showed a significant and nonlinear

Table 4.3 Nonlinear models of enriched ^{137}Ba mark transmission success for *D. aruanus* females injected with single injections of 0.5, 1 or 5 µg ^{137}Ba g^{-1} fish weight (treatment T0.5, T1 and T5 respectively). a) Results of the nonlinear mixed-effects model with treatment as fixed effect and colony as random effect. b) Results of the nonlinear model without treatment and colony effects. SE = standard error.

a)

Random effetcs	Variance		
α Colony	8.1956E-10		
β Colony	5.5797E-14		
Residual	0.6847		
Fixed effects	Estimate	SE	p-value
α T0.5(Intercept)	4.9523	0.6697	0.0000
α T1	0.7127	1.8004	0.6978
α T5	-1.2339	1.0188	0.2446
β T0.5(Intercept)	0.0215	0.0054	0.0012
β T1	0.0098	0.0151	0.5263
β T5	-0.0020	0.0105	0.8549
Number of Observations	26		
Number of Groups	6		

b)

Parameters	Estimate	SE	p-value
α	4.5316	0.4649	8.0700E-10
β	0.0218	0.0044	4.7400E-05
Residual Std. Error	0.9224		

Table 4.4 Generalized linear mixed-effects model of spawning success of *D. aruanus* females injected with single injections of 0.5, 1 or 5 µg ^{137}Ba g^{-1} fish weight (treatment T0.5, T1 and T5 respectively) or with monthly repeated injections of 0.5 µg ^{137}Ba g^{-1} fish weight (treatment T0.5rep) or NaCl (treatment TNaClrep). SE = standard error.

Random effetcs	Variance		
Colony	2.2677E-08		
Residual	0.9999		
Fixed effects	Estimate	SE	p-value
Control (Intercept)	-2.5039	0.2342	0.0000
Time	0.0002	0.0014	0.8890
TNaClrep	0.2877	0.2545	0.3015
T0.5	-0.1137	0.2759	0.6947
T0.5rep	0.1681	0.2602	0.5420
T1	-0.2850	0.2873	0.3596
T5	-0.1961	0.2812	0.5117
Number of Observations	2,340		
Number of Groups	12		

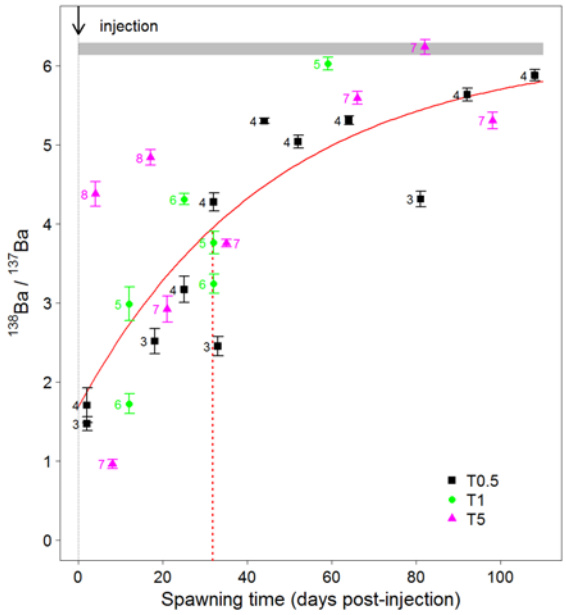

FIGURE 4.2 Mean (± standard error (SE)) $^{138}Ba/^{137}Ba$ ratios in otoliths of larvae produced by *D. aruanus* females injected once with $^{137}BaCl_2$ at dosage rates of 0.5 µg of ^{137}Ba g^{-1} fish weight (T0.5, black solid squares), 1 µg of ^{137}Ba g^{-1} fish weight (T1, green solid circles), 5 µg of ^{137}Ba g^{-1} fish weight (T5, magenta solid triangles), as a function of spawning time (days post-injection). The thick gray horizontal line represents mean (± SE) $^{138}Ba/^{137}Ba$ ratios in control otoliths. Numbers noted beside data points refer to the colony. Data points with error bars that overlap with thick gray bar are not significantly different from controls. Arrow indicates date of injection. Red curve represents best fit exponential decay relationship between time and Ba ratio for all single injection treatments. Red dash line indicates half-life for successful enriched Ba mark transmission.

effect of time on 1-day egg diameter and on 2-day larval SL (table 4.6). No significant effects of the different treatments were detected on 1-day egg diameter and on 2-day larval SL (table 4.6). The random factor colony explained 24% and 5% of variation in 1-day egg diameter and 2-day larval SL, respectively (table 4.6).

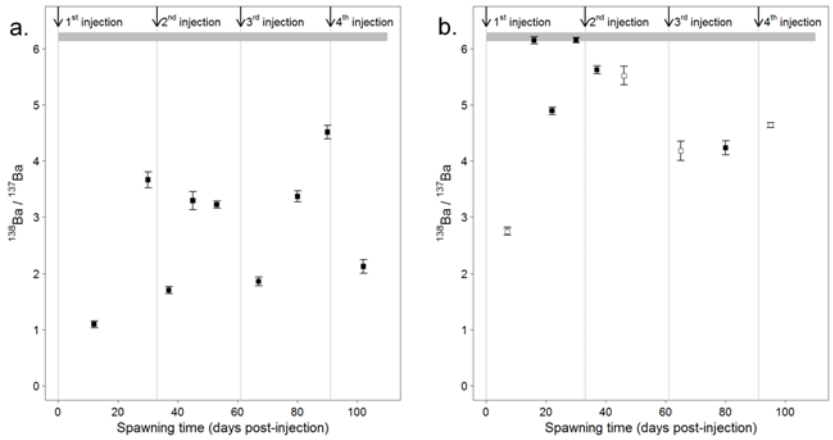

FIGURE 4.3 Mean (± standard error (SE)) $^{138}Ba/^{137}Ba$ ratios in otoliths of larvae produced by *D. aruanus* females from colonies 11 (a) and 12 (b) injected monthly with $^{137}BaCl_2$ at a dosage rate of 0.5 µg of ^{137}Ba g^{-1} fish weight (T0.5rep) as a function of spawning time (days post-injection). Black solid squares indicate samples analyzed using the standard beam diameter, whereas open squares are used for a few samples that were analyzed with a beam diameter of 25 µm. The thick gray horizontal line represents mean (± SE) $^{138}Ba/^{137}Ba$ ratios in control otoliths. Data points with error bars that overlap with thick gray bar are not significantly different from controls. Arrows indicate dates of injections.

TABLE 4.5 Number of spawning events, spawning lag (number of days between the first and the last observed spawning events in a colony) and spawning periodicity observed for each colony from the 25^{th} of October 2012 to the 7^{th} of May 2013. SD = standard deviation.

Treatment	Colony	Number of spawning events	Spawning lag (days)	Spawning periodicity (days)
Control	1	16	158	9.9
	2	14	132	9.4
T0.5	3	11	158	14.4
	4	16	186	11.6
T1	5	12	168	14.0
	6	11	117	10.6
T5	7	11	148	13.5
	8	14	91	6.5
TNaClrep	9	24	186	7.8
	10	15	158	10.5
T0.5rep	11	17	167	9.8
	12	18	189	10.5
			Mean	10.7
			SD	2.4

Table 4.6 Linear mixed-effects models of 1-day eggs diameter and 2-day larval standard length (SL) produced by *D. aruanus* females injected with single injections of 0.5, 1 or 5 µg $^{137}Ba\ g^{-1}$ fish weight (treatment T0.5, T1 and T5 respectively) or with monthly repeated injections of 0.5 µg $^{137}Ba\ g^{-1}$ fish weight (treatment T0.5rep) or NaCl (treatment TNaClrep). SE = standard error.

Random effetcs	(a) 1-day egg diameter (µm)			(b) 2-day larval SL (mm)		
	Variance			Variance		
Colony	150.6273			0.0250		
Residual	481.5505			0.1111		
Fixed effects	**(a) 1-day egg diameter (µm)**			**(b) 2-day larval SL (mm)**		
	Estimate	SE	p-value	Estimate	SE	p-value
Control (Intercept)	599.5250	8.7459	0.0000	2.6492	0.0194	0.0000
Time	1.5151	3.9917E-02	0.0000	-1.5678E-03	2.9517E-04	0.0000
$Time^2$	-2.7123E-02	5.1800E-04	0.0000	-3.1044E-07	3.6050E-06	0.9314
$Time^3$	1.0778E-04	2.0000E-06	0.0000	3.3822E-08	1.3000E-08	0.0088
TNaClrep	1.5504	12.2990	0.9038	-0.0290	0.0255	0.2977
T0.5	-0.2032	12.3053	0.9874	-0.0474	0.0255	0.1123
T0.5rep	-10.3367	12.2982	0.4328	-0.0511	0.0254	0.0909
T1	1.7710	12.3052	0.8903	-0.0205	0.0256	0.4527
T5	-15.2350	12.3013	0.2618	-0.0442	0.0255	0.1343
Number of Observations	10,804		9,771			
Number of Groups	12		12			

4.4 Discussion

4.4.1 Success of maternal transmission

Transgenerational isotope labeling of *D. aruanus* embryonic otoliths was experimentally tested for 44 spawning events ranging from 2 to 108 days post-injection of 0.5 to 5 µg ^{137}Ba g^{-1} fish weight, resulting in 41 clutches (or 93%) successfully marked (figs. 4.2 and 4.3). Irrespective of dosage, 100% of first spawning events after injection of females produced larvae with otoliths having significantly lower ^{138}Ba/^{137}Ba ratios (figs. 4.2 and 4.3). Mark transmission failure in 3 clutches spawned after the first spawning event may have resulted for a variety of reasons. Laser ablation may hit calcareous detritus instead of otoliths, and therefore fail to indicate marked individuals. Among the three clutches for which we were unable to detect any mark, two clutches displayed small calcareous detritus in the preparation (colony 12 at 16 days postinjection, and colony 7 at 82 days), suggesting possible failure due to contamination during otolith extraction as opposed to real negative marking. If this was the case, then the true mark success rate would increase from 93 to 98% (41 out of 42 correctly analyzed clutches). Alternatively, injected Barium solution in some females may have been leached via the dermal puncture wound or excreted via the intestinal tract (Roy et al. 2013). As females in an aquarium occasionally spawn asynchronously, this can lead to mark failure if a given clutch only represents a small number of individuals. This could explain the two failed mark transmission events in colony 12 after the initial injection. Finally, there may be individual differences in Barium metabolism among spawning females (Pecl et al. 2010), for example distinct Barium uptake in bone tissue (Roy et al. 2013). Such differences are supported by the occasional considerable variation in ^{138}Ba/^{137}Ba ratio between colonies that received the same treatment (e.g., first spawning of colonies 7 and 8, and colonies 5 and 6 ; fig. 4.2).

4.4.2 Longevity in maternal transmission and repeated injections

Besides validating the effective maternal transmission of enriched stable isotopes to embryonic otoliths, we assessed the time during which injected females produced marked larvae. Results indicate that isotope ratios remained clearly distinguishable in larval otoliths from those of control individuals for at least two months after initial injection for the intermediate and highest dosage (1 and 5 µg ^{137}Ba g^{-1} fish weight) and until 3.5 months for the lowest dosage tested (0.5 µg ^{137}Ba g^{-1} fish weight) (fig. 4.2). This is consistent with the results of Thorrold et al. (2006),who found 3-month longevity in maternal transmission for another Pomacentridae (*Amphiprion melanopus*) with a dose of 2.3 µg ^{137}Ba g^{-1} fish weight (section 4.6). Enriched Ba mark transmission follows an

exponential decaywith a half-life of 32 days with no difference among tested doses, suggesting that successful marking may be achieved for *D. aruanus* at a dose even lower than 0.5 µg $^{137}Ba\ g^{-1}$ fish weight.

One colony in the repeated injection treatment spawned four times during the month following the first injection and produced larvae whose otoliths were found to be significantly marked for the 1st and 3rd spawning events, but not for the 2nd and 4th events (colony 12 in fig. 4.3b). These results indicate that monthly repeated injections may be required to assure mark transmission and limit the impact of failed single injections when the aim of the study is to mark larvae over several months. Our repeated injection treatment indeed showed that each injection resulted in a decrease in $^{138}Ba/^{137}Ba$ ratios (fig. 4.3), generating persistent detection of the mark in all cases after the 2nd injection.

4.4.3 Impacts of marking on spawning success and condition of offspring

Before implementing transgenerational marking in the wild, it is imperative to investigate potential negative effects of the Barium injection on adult females and their offspring. Our results showed that neither single injections of any of the three Ba doses nor repeated injections of the lowest Ba dose had an effect on spawning success. This is consistent with the few other studies that have assessed the impact of transgenerational isotope labeling on adult females, indicating no negative effect on physiological stress (Roy et al. 2013, Williamson et al. 2009) or spawning success (Munro et al. 2009) (section 4.6). Regarding the effects on offspring, some studies assessing the effects of transgenerational marking using enriched Ba isotopes found positive (Starrs et al. 2013) or negative (Williamson et al. 2009) effects on newly hatched larvae depending on species and Ba doses (section 4.6). Size at hatching partly determines post-settlement survivorship (Vigliola and Meekan 2002, Vigliola et al. 2007) which is crucial in connectivity studies. Our results showed that the different treatments did not significantly affect 1-day egg diameter and 2-day larval standard length. Investigating impacts on growth throughout the larval period would also be worthwhile as negative effects have been detected for 3-week old larvae for a Pomacentridae (Roy (2008), section 4.6). However, this requires rearing larvae of *D. aruanus*, which is notoriously difficult (Danilowicz and Brown 1992, Gopakumar et al. 2009), as it is for most tropical reef fish larvae because of their small mouth gape (Moorhead and Zeng 2010).

4.4.4 Applications and implications for future studies

We developed a new method for extraction, laser ablation and analysis of otoliths of very young larvae (2-day old). Compared to previous studies that analyzed the core of single otoliths of juveniles that had reached the age of settlement (Huelga-Suarez et al. 2011, 2012, Munro et al. 2008, 2009, Thorrold et al. 2006, Williamson et al. 2009), the advantages of our method are (1) that the rearing of 2-day larvae requires reduced aquarium facilities and time, and (2) that rapid and simultaneous extraction and laser ablation of hundreds of otoliths is possible. In this study, we measured an average isotope ratio per clutch by ablating multiple otoliths at once, and thus we cannot state with certainty that all individuals originating fromthe same clutch are successfully marked. Nevertheless, we worked at the clutch level as we consider it to be very unlikely that the mark success rate of individual larvae originating from the same clutch differs significantly from 100% provided an effective dosage is used. Other studies investigating success of transgenerational marking found that maternal injections of effective dosages resulted in 100% mark success for juveniles originating fromthe same clutch (e.g., Williamson et al. (2009), Starrs et al. (2014)).

Nevertheless, it is worthwhile and feasible to evaluate the risk of miss-identifying marked fish by investigating the mark success rate at the individual level in future studies. A slightly modified version of our protocol could be used to analyze single otoliths of very young larvae, though such analyses may be technically more challenging. The bleaching dissolution technique may be used to extract individual otoliths by placing a single larva in a Petri dish, putting a few drops of bleach on it and waiting until the organic matter totally dissolves. In this case, the centrifugation and shaking processes that are necessary when dissolving multiple larvae would be unnecessary. Another possibility to allow for individual ablation of larval otoliths originating from the same clutch is to carefully separate aggregated otoliths with tweezers before the glue dries, creating sufficient space around otoliths so that each can be individually analyzed. In this study we used a laser beam diameter that was large enough to ablate several otoliths simultaneously to ensure that enough material was sent to the ICP-MS for the enriched ^{137}Ba to be detected. A smaller laser beam diameter that fits the size of a 2-day larva otolith (25 µm) must be used for laser ablation of an individual otolith and parameters like beam power and pulse rate must be adjusted to ensure that otolith material is correctly ablated. With these modifications, our method could therefore be used to determine a maternal transmission probability among individuals in a single clutch.

Based on our results, future studies investigating population connectivity of *D. aruanus* throughout an entire reproductive season should use monthly repeated in-

jections of 0.5 µg $^{137}Ba\ g^{-1}$ fish weight. We have shown that such treatments produce consistently significantly marked larvae over the reproductive season without negatively impacting spawning success or condition of eggs and larvae. Higher dosages are equally effective and show no signs of negative impacts on larval health, but will be more costly than the lower dosage.

Since the seminal study of Thorrold et al. (2006), transgenerational isotope labeling has proved to be a promising empirical method for tracking larvae for a range of marine and freshwater species. Differential marking by combining several different enriched isotopes (e.g., Huelga-Suarez et al. (2011, 2012), Starrs et al. (2014)) allows offspring to be clearly identified in connectivity studies investigating more than one source population. Even if the results of transgenerational marking are limited to snapshots in time and space and require significant field work, this method provides invaluable data to evaluate the performance of larval dispersal models and to contribute to the assessment of marine population connectivity and persistence (Almany et al. (2007), Burgess et al. (2014), Lett et al., under review).

4.5 Acknowledgments

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4.6 Supplementary material

Preparation of 25 ml of $BaCl_2$ stock solution at 5 mg Ba ml^{-1} :

- Weight 179.6 mg of $BaCO_3$ in a weighing scoop with a precision balance
- Place the $BaCO_3$ in a 25 ml graduated flask
- Add 9 ml ultrapure water
- Add 1 ml HCl at 36%
- Complete with ultrapure water until 25 ml mark

Preparation of 50 ml of $NaHCO_3$ solution at 54 mg ml^{-1} :

- Weight 2700 mg of $NaCO_3$ in a weighing scoop with a precision balance
- Place the $NaCO_3$ in a 50 ml graduated flask
- Complete with ultrapure water until 50 ml mark

Preparation of 50 ml of diluted solution at 50 µg Ba ml^{-1} for injection at 0.5 µg ^{137}Ba g^{-1} fish weight :

- Place 10 ml ultrapure water in a 50 ml graduated flask
- Add 0.5 ml of stock solution at 5 mg Ba ml^{-1} with a micropipette
- Add 1.5 ml of $NaHCO_3$ solution at 54 mg ml^{-1} with a micropipette
- Complete with ultrapure water until 50 ml mark

Preparation of 50 ml of diluted solution at 100 µg Ba ml^{-1} for injection at 1 µg ^{137}Ba g^{-1} fish weight :

- Place 10 ml ultrapure water in a 50 ml graduated flask
- Add 1 ml of stock solution at 5 mg Ba ml^{-1} with a micropipette
- Add 3 ml of $NaHCO_3$ solution at 54 mg ml^{-1} with a micropipette
- Complete with ultrapure water until 50 ml mark

Preparation of 50 ml of diluted solution at 500 µg Ba ml^{-1} for injection at 1 µg ^{137}Ba g^{-1} fish weight :

- Place 10 ml ultrapure water in a 50 ml graduated flask
- Add 5 ml of stock solution at 5 mg Ba ml^{-1} with a micropipette
- Add 15 ml of $NaHCO_3$ solution at 54 mg ml^{-1} with a micropipette
- Complete with ultrapure water until 50 ml mark

Injections :

Note that a volume of 0.05 ml g^{-1} of fish weight was defined as a limit volume of injected liquid that a fish could tolerate.

- Fish of 5 g : injection of 0.05 ml of diluted solution at 50, 100 or 500 µg Ba ml^{-1} to obtain Ba dosage of 0.5, 1 or 5 µg ^{137}Ba g^{-1} fish weight
- Fish of 10 g : injection of 0.1 ml of diluted solution at 50, 100 or 500 µg Ba ml^{-1} to obtain Ba dosage of 0.5, 1 or 5 µg ^{137}Ba g^{-1} fish weight
- Fish of 15 g : injection of 0.15 ml of diluted solution at 50, 100 or 500 µg Ba ml^{-1} to obtain Ba dosage of 0.5, 1 or 5 µg ^{137}Ba g^{-1} fish weight

Definition of three length categories and corresponding injected volume :

The end of our experimental tests on transgenerational isotope labeling was to finally apply this technique to wild populations of *Dascyllus aruanus*. Hence, the manipulation must be as simple as possible to implement on SCUBA. As it is easier to measure fish length instead of fish weight when working underwater, we focused on fish total length to determine which volume of solution had to be injected in order to obtain the desired Ba dosage. We then fixed three categories of injected volume based on fish total length (table 4.7).

Table 4.7 Fish weight (W, g), corresponding fork length (FL, cm) and total length (TL, cm) for *Dascyllus aruanus*, TL categories and injected volume of $BaCl_2$ diluted solution (ml) for each length category. FL was calculated from W using an allometric length-weight relationship ($W = 0.0716 * FL^{2.635}$; Letourneur et al. (1998)). TL was calculated from FL using a linear length-length relationship (TL = 1.075 * FL ; www.fishbase.org).

W(g)	FL (cm)	TL (cm)	TL categories	Injected volume of $BaCl_2$ diluted solution (ml)
1	2.7	2.9		
2	3.5	3.8	Category I	0.05
3	4.1	4.4	(TL $\leq$ 5.4 cm)	
4	4.6	4.9		
5	5	5.4		
6	5.4	5.8		
7	5.7	6.1	Category II	0.1
8	6	6.4	(5.4 $<$ TL $\leq$ 7 cm)	
9	6.3	6.7		
10	6.5	7		
11	6.8	7.3		
12	7	7.5	Category III	0.15
13	7.2	7.7	(TL $>$ 7 cm)	
14	7.4	8		
15	7.6	8.2		

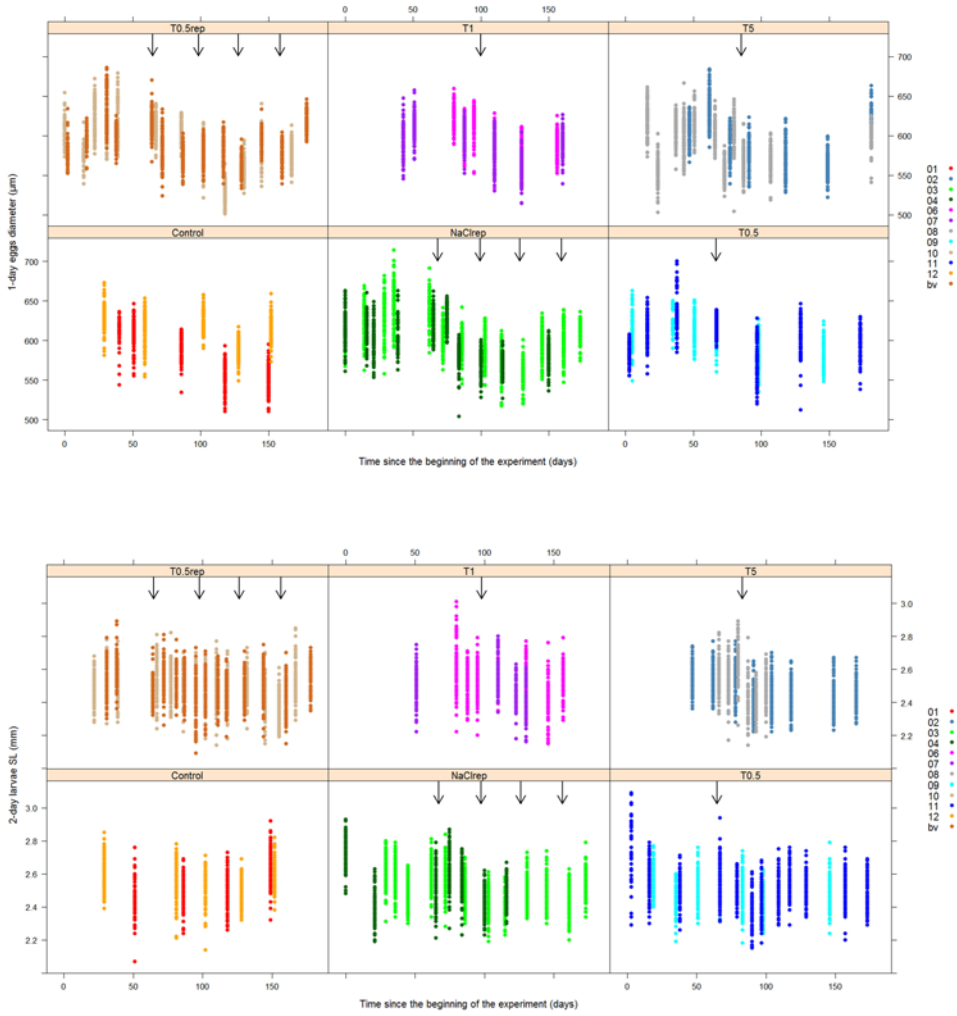

FIGURE 4.4 1-day egg diameter (A) and 2-day larval standard length (SL) (B) for clutches produced at various times since the beginning of the experiment, for the 12 colonies within 6 treatments. Arrows indicate dates of injections.

Table 4.8 Review of studies investigating transgenerational isotope labeling using injections of enriched ^{137}Ba. FW = fish weight. * indicates mixture of ^{135} and ^{137}Ba

References	Species	Dosage rate (µg ^{137}Ba g^{-1} FW)	Longevity in maternal transmission	Assessment of effects			
				Measured variables			Significant effects
				Adult females	Eggs	Larvae	
Thorrold et al. (2006)	Fire clownfish (*Amphiprion melanopus*)	0.45	$\geq$ 60 days	-	-	Hatching time Diameter of larval otoliths	No
		2.3	$\geq$ 90 days				
		4.5	$\geq$ 30 days				
		23	$\geq$ 60 days				
	Black seabass (*Centropristis striata*)	0.8	-				
Roy (2008)	Orange clownfish (*Amphiprion percula*)	0.5	$\sim$ 40 days	-	Clutch size Clutch area	Length of 1-, 14- & 21-d larvae Weight of 21-d larvae	Negative effect of the highest dose on 21-d larval length
		1 ; 2.5	$\geq$ 80 days				
Munro et al. (2009)	Golden perch (*Macquiria ambigua*)	20 ; 40	-	Spawning success	Fertilization rate	Hatching rate	No

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TABLE 4.8 – continued from previous page

References	Species	Dosage rate (µg ^{137}Ba g^{-1} FW)	Longevity in maternal transmission	Assessment of effects			
				Measured variables			Significant effects
				Adult females	Eggs	Larvae	
Williamson et al. (2009)	Coral-reef grouper (*Epinephelus fuscoguttatus*)	0.5 ; 2	-	Timing of spawning	Egg diameter Egg area	1-, 2- & 3-d larvae : SL ; Head depth ; Yolk sac area ; Oil globule area ; Eye diameter	Negative effect of the highest dose on 2-d larval SL and oil globule area and on 1-d larval yolk sac area ; Negative effect of both doses on 2-d larval head depth
Williamson et al. (2009)	Coral-reef grouper (*Plectropomus leopardus*)	2 ; 4	-	Whole blood variables ; Plasma concentrations of steroid hormones	-	-	No
Pecl et al. (2010)	Southern calamary (*Sepioteuthis australis*)	2.3 ; 4.5 ; 23	∼ 15 days	-	-	-	-
	Southern dumpling squid (*Euprymna tasmanica*)		∼ 35 days				
	Pale octopus *Octopus pallidus*)		0				

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Table 4.8 – continued from previous page

References	Species	Dosage rate (µg ^{137}Ba g^{-1} FW)	Longevity in maternal transmission	Assessment of effects			
				Measured variables			Significant effects
				Adult females	Eggs	Larvae	
	Maori octopus *Octopus maorum*)		0				
Huelga-Suarez et al. (2011)	Brown trout (*Salmo trutta*)	0.3*	≥ 7 months	-	-	-	-
Huelga-Suarez et al. (2012)	Atlantic salmon (*Salmo salar*)	0.3*	-	-	-	-	-
Starrs et al. (2013)	Eastern rainbowfish (*Melanotaenia splendida*)	20 ; 40	≥ 6 months	-	-	1-day larvae : Hatching time ; SL ; Body depth ; Eyeball diameter	Positive effect of the lowest dose on larval SL, body depth, and eyeball diameter
Starrs et al. (2014)	Purple-spotted gudgeon (*Mogurnda adspersa*)	20	-	-	-	1- & 12-d larvae : SL ; Body depth ; Eyeball diameter ; 31-d larvae : TL	Negative effect on larval body depth
Roy et al. (2013)	Fire clownfish (*Amphiprion melanopus*)	2 ; 4	56 days *in female gonad*	Plasma cortisol concentration	-	-	No

Chapitre 5

Monthly variability of self-recruitment for a coral reef damselfish

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Abstract

Understanding the dynamics of marine populations is critical to manage marine systems effectively, and requires information on patterns and scales of population dispersal and connectivity that are still poorly known. We used artificial transgenerational marking of embryonic otoliths to study larval dispersal of the Humbug damselfish, *Dascyllus aruanus*, in the patchy reefs seascape of the South-West Lagoon of New Caledonia (SWL), South-West Tropical Pacific. The adult population of a patch reef located in the central part of the SWL was injected repeatedly with an enriched ^{137}Ba solution to ensure mass production of marked larvae over two successive reproductive seasons. Multiple cohorts of newly settled larvae were sampled and their otolith core analyzed by laser-ICPMS to assess the seasonal and interannual variability of self-recruitment at the central reef. Connectivity between this reef and ten neighboring reefs was also estimated. Analysis of 1,200 settlers indicates that self-recruitment varies significantly between months (ranging from 0% to 68%) and years (21% in 2011 and 0% in 2012). However, variable self-recruitment did not always correspond to variable numbers of self-recruits. Therefore, whereas self-recruitment is undoubtedly a good indication of population openness, it may not elucidate local population persistence. Finally, being the first self-recruitment study to include such a large number of settlers, our work reveals that the threshold used to determine marked individuals significantly affects perceived self-recruitment and connectivity rates, and, therefore, must be carefully chosen.

Keywords : *Dascyllus aruanus*, Transgenerational marking, Otolith, Self-recruitment, Larval dispersal, Connectivity

5.1 Introduction

Most coastal marine species begin their life cycle with a planktonic larval stage (Leis et al. 2013). During this stage, larvae may be dispersed to distant populations, retained within their natal population or lost in the marine realm (Jones et al. 2009). Knowledge of larval connectivity/retention within spatially-distributed marine populations is crucial for understanding population dynamics, persistence, and resilience, and to implement effective management measures such as Marine Protected Areas (Sale et al. 2005, Botsford et al. 2009, Burgess et al. 2014). However, the quantification of larval connectivity and retention is a very difficult task given the complexity of directly observing a multitude of tiny larvae subject to high mortality rates in the immensity of the ocean (Thorrold et al. 2001). Moreover, larval dispersal may be influenced by many factors including oceanic currents, pelagic larval duration (PLD), larval behavior and the distribution of suitable habitat (Sponaugle et al. 2002, Gerlach et al. 2007, Pinsky et al. 2012).

It was historically assumed that large scale dispersal of larvae was the rule in marine systems (Caley et al. 1996). This paradigm has been challenged over the past 15 years, with studies suggesting shorter dispersal distances (100 m to 100 km) than originally suspected and consequently higher self-recruitment for some species (e.g., Jones et al. (1999), Cowen et al. (2000), Almany et al. (2007)). Among the empirical techniques for estimating larval connectivity, transgenerational marking of calcified structures (Thorrold et al. 2006, Hobbs et al. 2012) has proved effective when the environment is chemically too homogenous to use natural chemical signatures (Almany et al. 2007). This mass marking technique relies on the transmission of a permanent and artificial chemical mark (e.g., via injection of water enriched in a particular stable isotope) from gravid females to their offspring. Larvae or juveniles are later captured and the original populations of marked individuals are identified by analyzing the chemical composition of calcified structures (e.g., otoliths for fish). To our knowledge, despite validation in captivity for a range of fish species (e.g., Thorrold et al. (2006), Munro et al. (2009), Starrs et al. (2014), Cuif et al. (2014)), this method has been applied only once in the field (Almany et al. 2007).

Empirical studies of self-recruitment and connectivity are limited in number and often restricted to single dispersal events (Jones et al. 1999, Almany et al. 2007, D'Aloia et al. 2013, Schunter et al. 2014). However, investigating the temporal variability of dispersal patterns at several seasonal and interannual scales is essential for understanding the degree of openness of marine populations and thus inferring sizing and spacing of marine protected area networks (Selkoe et al. 2006, Pusack et al. 2014). Among the few studies investigating such temporal variability, most focused on interannual

variability, via sampling realized over 2 to 6 years (Jones et al. 2005, Carreras-Carbonell et al. 2007, Carson et al. 2010, Hogan et al. 2012, Saenz-Agudelo et al. 2012). Only a limited number of studies provide an assessment of intra-seasonal variability (Planes et al. 2009, Carson et al. 2010, Madduppa et al. 2014). At both temporal scales, studies showed either remarkable stability of self-recruitment and/or connectivity over time (Carreras-Carbonell et al. 2007, Planes et al. 2009), or a significant temporal variability (Hogan et al. 2012). To our knowledge, the only studies estimating self-recruitment and connectivity variability at a monthly scale also revealed significant variability (Chittaro and Hogan 2013, Cook et al. 2014). Recent biophysical modelling work also suggests large temporal variability in larval dispersal patterns (Cuif et al. 2014) emphasizing the need for empirical data to corroborate these results.

The objective of this study is to quantify inter-annual and monthly variability in larval dispersal of the Humbug damselfish *Dascyllus aruanus* (Linnaeus 1758) in the patchy reefs seascape of the South-West Lagoon of New Caledonia (SWL) using transgenerational marking with enriched stable isotope of ^{137}Ba. *D. aruanus* provides an excellent model for studying variability in larval dispersal patterns because of its high density in the SWL, its strong territoriality after settlement that limits dispersal of adults among local populations and its significant potential for larval transport due to its 3 week Pelagic Larval Duration (PLD ; Wellington and Victor (1989)). Furthermore, the SWL, located in the South-West Tropical Pacific 1500 km east of Australia, is regularly flushed by strong trade winds (Douillet 1998), potentially producing large temporal variability in larval dispersal and recruitment (Cuif et al. 2014). Our results provide the first direct estimates of spatio-temporal variability in self-recruitment and connectivity in the SWL. These estimates are critical for the validation of simulation-based predictions of larval dispersal patterns ultimately supporting marine resources management.

5.2 Methods

5.2.1 Study species and area

The Humbug damselfish *D. aruanus* (Linnaeus 1758) is a batch spawner (Fricke and Holzberg 1974) that lives among branching coral in spatially discrete groups (Holbrook et al. 2000). To study larval transport of this species, a focal reef was chosen for its central position in the SWL (22.2951 °S - 166.3322 °E) and the reasonable size of the *D. aruanus* population living there (fig. 5.1a, b). *D. aruanus* branching coral colonies were found on the reef flat ($\sim$ 3 m depth), the fore reef (from $\sim$ 3 to 10 m depth) and on the surrounding sandy bottom (from $\sim$ 10 to 12 m depth) with the exception of the

north-east quarter of the reef where sea grass beds dominated (fig. 5.1b). The focal reef was surveyed in totality by scuba divers from late October to early November 2011 to count all *D. aruanus* colonies (508 in total, fig. 5.1b). Every colony was labeled with a number carved on a squared piece of plastic attached to the coral with a Colson strip (fig. 5.1c). To facilitate field work, the reef was then subdivided into 10 zones comprising approximately 50 colonies each. A subsample of 100 colonies was generated by randomly selecting 10 colonies in each zone (fig. 5.1b). Ten reefs located at various distances (range 3 - 20 km) and directions from the focal reef were selected in order to estimate larval connectivity between the focal reef and surrounding areas (fig. 5.1a).

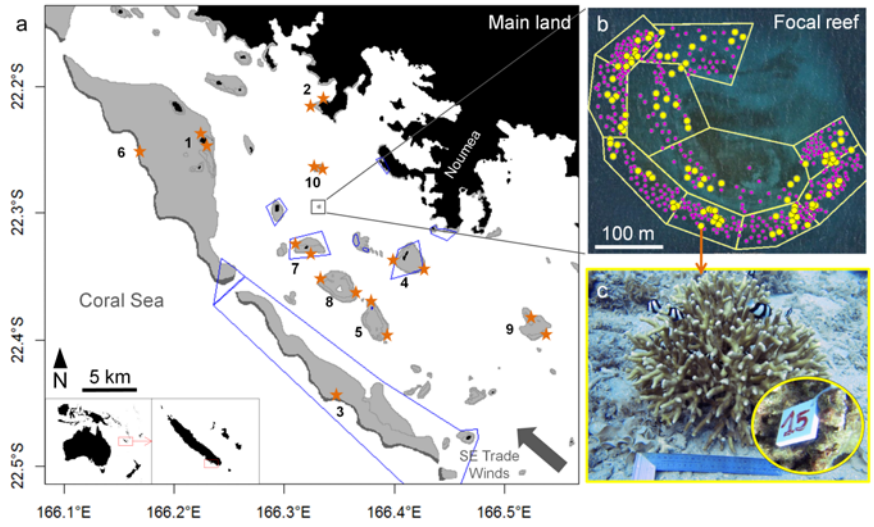

FIGURE 5.1 Study area. a) The South-West Lagoon of New Caledonia (SWL) : localization of the focal reef and the 10 neighboring reefs (numbered from 1 to 10). Land is depicted in black, barrier reef in dark grey and shallow reefs in light grey. Orange stars indicate juveniles sampling stations on the 10 neighboring reefs. Blue polygons localize Marine Protected Areas. b) Focal reef divided into 10 subareas containing $\sim$ 50 colonies each. Pink and yellow points indicate approximate localization of the 508 focal reef *Dascyllus aruanus* colonies and localization of the 100 colonies randomly selected for injections. c) Adults *D. aruanus* on their branching coral colony and zoom on a colony numbered tag.

5.2.2 Transgenerational marking

The transgenerational marking procedure used in this study is fully described in Cuif et al. (2014) where it was shown to be an effective means of mass marking *D. aruanus* larvae without any adverse effects on females, eggs and hatchlings. Barium carbonate ($BaCO_3$) highly enriched in stable ^{137}Ba isotope (81.9%) and depleted in

^{138}Ba (17.4%) as compared to natural Ba isotope (11.32% ^{137}Ba and 71.66% ^{138}Ba) was prepared in solution. Fish were tagged underwater *in situ* by scuba divers over a 2-week period each month over two reproductive seasons from November 2011 to January 2012, and from December 2012 to January 2013 (table 5.1). Two divers approached the colony so that fish hide among branches. The branching coral was then surrounded with a large plastic bag and a small volume of alcoholic clove-oil solution (5%) was injected under the bag to anesthetize all fish. Each fish was then captured, placed in a net bag, measured and, if total length (TL) was superior to 30 mm (i.e., potential reproducer, unpublished data), injected in the abdominal cavity with the appropriate volume of ^{137}Ba solution at a dose of 0.5 µg of ^{137}Ba g^{-1} fish weight using a 0.3 ml insulin syringe. As males and females were not distinguishable, all mature fish were injected. Fish were then released all together on their coral colony. As the half-life of Ba transmission from female to offspring is approximately one month at the chosen dose (Cuif et al. 2014), we repeated injections monthly to ensure that all larvae produced during the study duration were marked. The same colonies were tagged each month on the focal reef (fig. 5.1b, table 5.1). The percentage of colonies injected with ^{137}Ba solution each month was estimated as 19.7% except in December 2011 where it was 18.5% (table 5.1). We assumed that this percentage represented the fraction of marked adults in the focal population.

Table 5.1 Injections dates of *Dascyllus aruanus* colonies on the focal reef, number of colonies injected each month and corresponding percentage of focal population marked. Settlement dates intervals and assigned settlement months.

Injections dates	Number of injected colonies	% focal population marked	Settlement dates	Settlement month
21 Nov. to 2 Dec. 2011	100	19.7	25 Dec. to 20 Jan. 2012	J12
19 to 30 Dec. 2011	94	18.5	21 Jan. to 20 Feb. 2012	F12
16 to 27 Jan. 2012	100	19.7	21 Feb. to 20 Mar. 2012	M12
Total year 1	294	19.3		
10 to 19 Dec. 2012	100	19.7	11 Jan to 11 Feb. 2013	F13
7 to 17 Jan. 2013	100	19.7	12 Feb. To 11 Mar. 2013	M13
Total year 2	200	19.7		
Total years 1 & 2	494	19.4		

5.2.3 Settlers and juveniles sampling

On the focal reef, random sampling of settlers (< 15 mm TL) was performed by scuba divers from December 2011 to March 2012, and from December 2012 to March 2013. Settlers from December 2012 were used as controls. Between 69 and 175 settlers were collected each month totaling 721. On the ten reefs surrounding the focal reef, a

total of 941 juveniles (< 20 mm TL) covering 3 larval cohorts from January to March 2012 were also collected in March 2012, with approximately 100 juveniles at each reef. Except for barrier reef stations where only one site was sampled, all reefs were sampled on both leeward and windward sides, with approximately 50 juveniles collected on each side (fig. 5.1a). Sampling was performed at approximately 10 meters depth on each reef. Settlers (or juveniles) were anesthetized using a spray of alcoholic clove-oil solution (5%), captured with hand nets, transported to the surface in plastic bags and killed by cold shock. Upon return to the laboratory, samples were immediately placed in a freezer.
Settlement on the focal reef (number of settlers per colony) was estimated each month by counting the number of settlers on a random sample of colonies (table 5.4).

5.2.4 Otoliths analysis

Preparation

The total length of each individual was measured with electronic calipers to the nearest 0.1 mm before otolith extraction. Both sagittal otoliths were extracted under a binocular microscope by dissecting the dorsal section of the skull using scalpel and tweezers. Otoliths were cleaned in ultra pure water to remove adhering tissue, dried in a laminar flow hood for 24 h and stored in a 0.5 ml microcentrifuge tube. One sagittal otolith per individual was then mounted rostrum up in thermoplastic glue (Crystalbond 509) on a piece of microscope slide (7.5 mm x 5 mm) affixed on a standard microscope slide with thermoplastic glue. The otolith rostrum was first grounded and polished and the otolith was then turned over, grounded to the mid-plane and polished with P1200 sandpaper and 9, 3 and 1 µm lapping film. A distance of approximately 20 µm was left between the polished surface and the core of the otolith to ensure that the entire core was ablated during ICP analysis. The piece of microscope slide containing the otolith section was unglued from the standard slide, rinsed in ultrapure water, dried in a laminar flow hood for 24 h and stored individually in 1.5 ml microcentrifuge tubes.

Age reading and PLD determination

Age at capture and PLD (in days) was estimated for a subsample of 502 settlers of the focal reef collected from December 2011 to March 2013. The number of daily increments was counted along the longest axis of the otolith from the core to the edge using the multipoint tool of the software program ImageJ (`http://rsbweb.nih.gov/ij/`). Wilson and McCormick (1999) showed that settlement mark for Pomacentridae consists of an abrupt decrease in increment width. PLD was hence determined for each fish as

the age at which increment width fell. Settlement date of each fish was calculated as capture date minus number of days since settlement.

In order to assign a settlement month to the focal reef's settlers for which age and PLD were not available, we adjusted a linear regression between size at capture and the number of days elapsed between settlement and capture for the 502 settlers whose age was known (fig. 5.4). It was then possible, from the size at capture and date of capture of a settler, to estimate its settlement date. For each reproductive season, we considered only settlers that settled in a time interval ranging from the date of the last injection performed at the beginning of the reproductive season plus the mean PLD and the date of the last injection performed at the end of the reproductive season plus the mean PLD, to ensure that all settlers collected were potentially issued from marked females. Then this time interval was divided into approximately month-long periods (table 5.1).

ICP-MS analysis

A New Wave Research 213 nm UV laser ablation system connected to an Agilent 7500cs inductively coupled plasma mass spectrometer (ICP-MS) located at the Charles Darwin University (Darwin, Australia) was used for the otolith elemental analysis. Otoliths were fixed per group of approximately 15 using double-sided tape on petrographic slides to be placed in the ablation chamber. A reference standard (NIST612, National Institute of Standards and Technology) was analyzed at the beginning and end of each $\sim$ 1-hour session to account for mass bias and machine drift throughout the analyses. Otoliths were vertical-depth profiled by laser ablation with a laser beam diameter of 30 µm and energy density of 7 to 11 J cm^{-2} pulsed at 5 Hz. Each analysis lasted 60 s including 10 s of background and 50 s of ablation (laser activated). If the otolith core was partly altered during grounding, a horizontally laser line scan (1 µm s^{-1}) was preferably undertaken across the otolith core. Each otolith core was analyzed for ^{43}Ca, ^{55}Mn, ^{137}Ba and ^{138}Ba (table 5.5).

Statistical analysis

All statistical analyses were conducted using R-2.15.0. ICP-MS data in the blank were first cleaned of outliers, defined as any value greater than 1.5 times the interquartile distance (Tukey 1977). Isotope abundances of ^{43}Ca, ^{55}Mn, ^{137}Ba and ^{138}Ba measured in each otolith were then blank-subtracted. We used the peak of ^{55}Mn as an indicator of the otolith core (Brophy et al. 2004, Macdonald et al. 2008, Munro et al. 2009, Starrs et al. 2014). To do this, counts of ^{43}Ca and ^{55}Mn were smoothed using a 5-point running mean to remove noise (Sinclair et al. 1998), after which the ratio

$^{55}Mn/^{43}Ca$ was computed. A peak of Mn was identified when the $^{55}Mn/^{43}Ca$ value rose above the modal value (calculated for each ablation) by a factor of 2 or more and stayed at this elevated level for a period of at least 2.5 s (corresponding to 3.5 data points, adapted from Brophy et al. (2004)). The mean core diameter of *D. aruanus* is approximately 15 µm (first increment width = 7.5 µm ± 1.2 standard deviation (SD), n = 502 fish) which corresponds to 21 data points in each ablation profile. These 21 data points centered on the ^{55}Mn peak were considered as measurements in the otolith core. ^{137}Ba and ^{138}Ba data in this core were cleaned of outliers and the mean $^{138}Ba/^{137}Ba$ ratio was then computed and corrected for mass bias using the reference standard in which the $^{138}Ba/^{137}Ba$ ratio was assumed to be the same as the relative natural abundance (i.e., 6.38) based on the International Union of Pure and Applied Chemistry values of isotopes abundance (Berglund and Wieser 2011). Controls were assayed throughout the ICP-MS runs.

A threshold on the mean $^{138}Ba/^{137}Ba$ ratio value inside the otolith core was determined below which an individual was considered marked. This threshold was equal to the mean $^{138}Ba/^{137}Ba$ ratio of the controls minus q * SD with q the quantile of the normal distribution corresponding to an adjusted p-value. An overall significance level (α) of 0.01 was chosen for statistical tests, which was then adjusted using the method of Benjamini and Yekutieli (2001) to control for the false discovery rate due to repeated statistical tests. The mean $^{138}Ba/^{137}Ba$ ratio in the control otoliths was 6.46 with a SD of 0.15 (n = 64). The final adjusted p-value was 1e-06, corresponding to q = 4.70 and therefore a $^{138}Ba/^{137}Ba$ threshold of 5.76 (table 5.2).

To test the sensitivity of our results to this particular threshold value, we also estimated the number of marked individuals for three other threshold values ranging from 5.65 to 6.16 (table 5.2). Two of these threshold values were based on the controls' mean ratio minus 2 or 3 times the controls ratios' SD (e.g., Almany et al. (2007), Munro et al. (2009)), corresponding approximately to α equal to 0.05 or 0.01 without adjustment and producing $^{138}Ba/^{137}Ba$ thresholds of 6.16 and 6.02 (table 5.2). The most conservative threshold value tested was calculated based on the difference in $^{138}Ba/^{137}Ba$ ratio between the mean of the controls and the maximum ratio observed in all recruits (7.27). This difference was then subtracted from the mean ratio, producing a $^{138}Ba/^{137}Ba$ threshold of 5.65 ("inverse max" threshold in table 5.2).

Connectivity between focal reef and reef j is here defined as the fraction of settlers on reef j that originated from the focal reef as :

$$C_j = \frac{m_j/s_j}{pm} \tag{5.1}$$

Table 5.2 Number of *Dascyllus aruanus* marked settlers found on the focal reef and on the ten neighboring reefs (Reefs 1 to 10) in function of threshold value. SD = standard deviation.

		Threshold			
	Settlement month	6.16 (2*SD)	6.02 (3*SD)	5.76 (α = 0.01, corrected, default)	5.65 (inverse max)
Focal reef	J12	8	1	0	0
	F12	10	3	2	2
	M12	30	21	11	9
	F13	11	1	0	0
	M13	9	4	0	0
	Total	68	30	13	11
Reef 1	J12 to M12	1	0	0	0
Reef 2	J12 to M12	1	0	0	0
Reef 3	J12 to M12	1	0	0	0
Reef 4	J12 to M12	5	2	0	0
Reef 5	J12 to M12	0	0	0	0
Reef 6	J12 to M12	1	0	0	0
Reef 7	J12 to M12	2	0	0	0
Reef 8	J12 to M12	1	0	0	0
Reef 9	J12 to M12	1	0	0	0
Reef 10	J12 to M12	0	0	0	0
	Total	13	2	0	0

with m_j the number of marked settlers on reef j, s_j the total number of settlers on reef j that were analyzed and pm the fraction of marked adult population on the focal reef. Self-recruitment on the focal reef was calculated similarly with j being the focal reef.

Self-recruitment and connectivity estimates are subject to uncertainty due to small sample size and the random nature of collecting marked versus unmarked individuals. 95% confidence intervals for self-recruitment and connectivity estimates were determined using a bootstrap approach assuming collection of marked settlers is the result of two random binomial processes : first, a collected recruit must be from the focal site ; second, collected recruits from the focal site must come from eggs that were marked. The probability of the first process is given by the fraction of all recruits that originated at the focal site, i.e., the self-recruitment or connectivity values that we wish to estimate. The probability that an egg produced at the focal site is marked (i.e., the second process) is assumed to be the same as the fraction of the total adult population injected with barium (table 5.1). Confidence intervals were calculated by randomly generating a large number of simulated recruitment samples and determining the likelihood that a potential self-recruitment or connectivity value will result in a sample

with the observed number of marked individuals given the known fraction of marked eggs produced at the focal site.

Mean PLD were compared by pairs using t-tests with a 95% confidence level.

5.3 Results

5.3.1 Self-recruitment and connectivity

A total of 1,249 otoliths presented a clear ^{55}Mn peak and were thus selected for self-recruitment and connectivity analyses (table 5.2 and table 5.3, fig. 5.5), including 564 otoliths collected on the focal reef, 685 on the ten neighboring reefs, and 64 for control.

Among these, a total of 13 otoliths were identified as marked with a ^{138}Ba/^{137}Ba threshold value of 5.76 (fig. 5.2, table 5.2 and table 5.3). All marked otoliths belonged to settlers collected on the focal reef with settlement dates ranging from February 13th to March 19th 2012 (table 5.6). Among these 13 self-recruits, 2 were assigned to February 2012 and 11 to March 2012 (table 5.3, table 5.6). Most of them were spawned and settled on two distinct events, with a first group of 4 self-recruits spawned around the 5-7 February and settled on the last week of February, and a second group of 5 self-recruits spawned around the 15-16 February and settled on 6-8 March (table 5.6).

Self-recruitment rates were 0% in January 2012, February 2013 and March 2013, 9% in February 2012 and considerably higher in March 2012 with 68% (table 5.3, fig. 5.3). Considering uncertainty due to sample size, it is 95% probable that self-recruitment was less than 12% in January 2012, 9% in February 2013, 13% in March 2013, and between 3 and 32% in February 2012, and between 39 and 97% in March 2012 (table 5.3). Overlapping confidence intervals revealed null to chronically low self-recruitment rates at all months except in March 2012 (fig. 5.3). Overall self-recruitment was estimated at 21% (12-35% CI) in 2012 and 0% ($<$ 6%) in 2013. Over the two reproductive seasons, self-recruitment was 12% with a 95% CI ranging from 7 to 19% (table 5.3).

No marked larvae were found on the ten neighboring reefs. Taking into account uncertainty due to sample size, we estimated that there is a 95% probability that connectivity was less than 2% between the focal reef and the 10 neighboring reefs, but could be up to 17-31% on any given reef (table 5.3).

Table 5.3 Number of *Dascyllus aruanus* settlers collected on the focal reef and on the 10 neighboring reefs (Reefs 1 to 10) that were analyzed with LA-ICP-MS for each settlement month and presented a clear ^{55}Mn peak in otolith core, number of marked settlers with the default threshold of 5.76, fraction of settlers on reef j that originated from the focal reef (C_j) and 95% confidence interval (CI).

Reef j	Settlement months	Number of otoliths with a ^{55}Mn peak	Number marked	C_j (%)	95% CI
Focal reef	J12	121	0	0	<12%
	F12	115	2	9%	[3% ; 32%]
	M12	82	11	68%	[39% ; 97%]
	Total year 1	318	13	21%	[12% ; 35%]
	F13	155	0	0	<9%
	M13	91	0	0	<16%
	Total year 2	246	0	0	<6%
	Total years 1 & 2	564	13	12%	[7% ; 19%]
Reef 1	J12 to M12	81	0	0	<18%
Reef 2	J12 to M12	51	0	0	<28%
Reef 3	J12 to M12	75	0	0	<19%
Reef 4	J12 to M12	84	0	0	<17%
Reef 5	J12 to M12	76	0	0	<18%
Reef 6	J12 to M12	70	0	0	<21%
Reef 7	J12 to M12	80	0	0	<18%
Reef 8	J12 to M12	77	0	0	<20%
Reef 9	J12 to M12	46	0	0	<31%
Reef 10	J12 to M12	45	0	0	<31%
	Total year 1	685	0	0	<2%

These results changed significantly when we changed the threshold for defining an individual as marked (table 5.2). For example, with the least conservative threshold (2*SD threshold), 68 individuals were identified as marked on the focal reef (instead of 13) over 2 reproductive seasons with a maximum of 30 marked individuals (instead of 11) in March 2012 and a minimum of 8 (instead of 0) in January 2012 (table 5.2, fig. 5.3).With this threshold, 13 marked individuals (instead of 0) originating from the focal reef were found to have settled on the neighboring reefs, especially on reef 4 located in the south-east. On the contrary, with the most conservative threshold ("inverse max" threshold) we obtained 11 marked individuals on the focal reef and zero on the neighboring reefs.

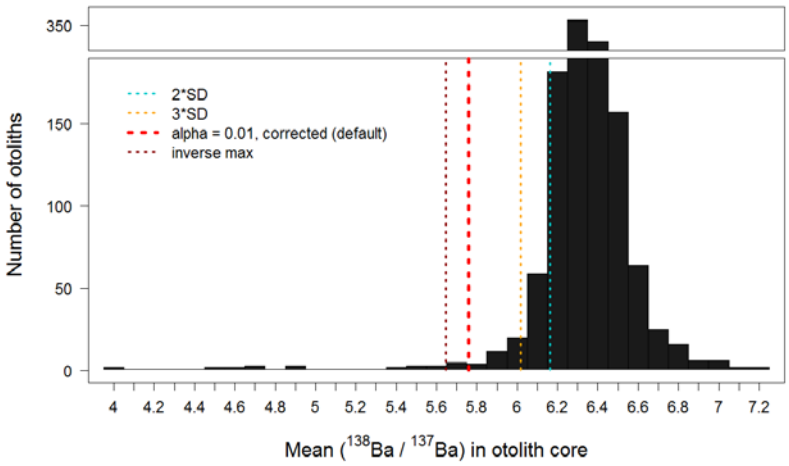

FIGURE 5.2 Frequency histogram of mean Barium ratio measured in *Dascyllus aruanus* otoliths cores (n = 1249). Values on the Y axis between 190 and 330 were omitted to reduce the gap in the range of values represented as bar heights. Vertical dotted lines indicate threshold values of 6.16 (2*SD, in cyan), 6.02 (3*SD, in orange), 5.76 (alpha = 0.01, corrected (default), in red) and 5.65 (inverse max, in dark red).

5.3.2 Settlement intensity and PLD

The mean number of settlers per colony on the focal reef ranged from 0.4 ($\pm$ 1.0 SD) in March 2012, to 6.8 ($\pm$ 6.9 SD) in February 2012 (fig. 5.3). These numbers corresponded to a total number of recruits to the focal reef population ranging from 305 in March 2012 to 3,454 the month before (table 5.4). Collected settlers had a mean age at capture of 36.4 days ($\pm$ 7.2 SD, n=502) and a mean PLD of 23.0 days ($\pm$ 3.4 SD). Mean PLD of settlers ranged from 20.7 days $\pm$ 2.1 SD (n = 64) in March 2012 to 23.6 days $\pm$ 2.9 SD (n = 76) in January 2012 (fig. 5.3). Mean PLD in March 2012 was significantly lower than the mean PLD of settled larvae the other months (fig. 5.3, table 5.7).

5.4 Discussion

A critical aspect of empirical methods assigning individuals to their population of origin is to identify with certainty as many truly marked individuals as possible, while minimizing false assignments (Christie et al. 2013, Harrison et al. 2013b,a). In transgenerational marking studies, it is common to use a threshold below which an individual is considered marked based on the $^{138}Ba/^{137}Ba$ ratio measured in the

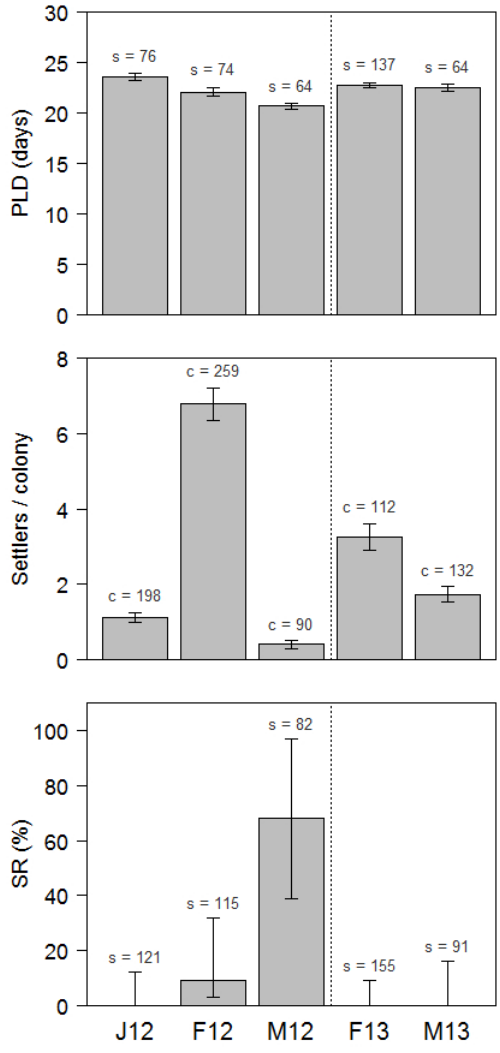

Figure 5.3 Mean PLD (in days) ± standard error (SE) of *Dascyllus aruanus* settlers for which otolith was read, mean number of *D. aruanus* settlers per colony on the focal reef ± SE, and *D. aruanus* self-recruitment rate (SR) ± 95% confidence interval for each settlement month on the focal reef. Numbers of settlers (s) and numbers of colonies (c) used to estimate each value are indicated above each bar.

core of control samples (e.g., Thorrold et al. (2006), Almany et al. (2007)). Our study demonstrates the importance of carefully choosing this threshold as results may be

quite sensitive to this value (fig. 5.2, table 5.2). Threshold values commonly used in the literature based on the controls' mean ratio minus 2 or 3 times the controls ratios' SD (e.g., Almany et al. (2007), Munro et al. (2009)) appear not be strict enough in our case, leading to false positives and subsequent overestimation of self-recruitment and connectivity among reefs. Plotting the distribution of mean Ba ratios for 1200 fish otolith cores, one finds that values of 6.16 (2*SD) or 6.02 (3*SD) are not unusual, lying well within a continuous distribution of sample values approximately normally distributed above and below the natural isotope ratio (fig. 5.2). To avoid misidentification of marked settlers, we used a statistical test that explicitly corrects for repeated measures (Benjamini and Yekutieli 2001). The resulting threshold value (5.76) thus accounts for potential overlapping of marked and unmarked individuals and avoids significant overcounting of marked individuals. Moreover, among the 13 individuals identified this way, most individuals (11) were considerably further from the natural mean than the maximum value is from that mean, which increases our confidence in our results (fig. 5.2, table 5.2). Overall, one needs to find an acceptable trade-off between false positives and negatives by testing sensitivity of results to several marking thresholds. In order to avoid false positives and inflate artificially self-recruitment and connectivity estimates, we recommend (1) to analyze as many control samples as possible because the threshold will be based on these, (2) to use a strong statistical power, (3) to adjust the significance level to control for the false discovery rate due to repeated statistical tests, and (4) to do a close examination of otolith profiles (e.g., fig. 5.5) to validate the assignment tests.

Our study revealed that self-recruitment of *D. aruanus* could occur in the SWL of New Caledonia on a small central patch of reef despite average water residence times (11 days, Jouon et al. (2006), Ouillon et al. (2010)) well below the PLD (23 days) of our study species. This PLD is typical of many tropical marine fishes (Victor and Wellington 2000, Lester and Ruttenberg 2005), and intermediate compared to other species investigated in similar larval connectivity studies (e.g., Saenz-Agudelo et al. (2012), Harrison et al. (2012)). We found an average self-recruitment of 12% over 2 reproductive seasons. This value is intermediate compared to results of other fine-scale studies using direct methods (transgenerational marking and genetic parentage analysis) with self-recruitment ranging from 0% (Saenz-Agudelo et al. 2012) to 65% (Berumen et al. 2012). Our intermediate value could be due to the intermediate PLD of our study species as high (low) self-recruitment rates have been reported for species with relatively short (long) PLDs (e.g., Planes et al. (2009), D'Aloia et al. (2013) ; but see Saenz-Agudelo et al. (2012), Berumen et al. (2012)). Besides PLD, habitat patchiness also influences the level of self-recruitment (Pinsky et al. 2012) with low self-recruitment estimated in continuous seascapes (distances between sites inferior to 2 km, D'Aloia et al. (2013), Schunter et al. (2014)) and high self-recruitment on isolated islands

(nearest reef at more than 10 km, Almany et al. (2007), Planes et al. (2009), Berumen et al. (2012)). In that context, the intermediate patchiness of the habitat in the SWL, where the closest reefs to our focal reef are approximately 3 km away, could also explain the intermediate value of self-recruitment that we found.

The significant interannual (21% in 2012, 0% in 2013) and intermonth (0% to 68%) variability of self-recruitment may be due to many factors, including wind-driven circulation in the SWL, cohesive larval dispersal and/or maternal age effects. Water circulation in the SWL is determined by strong and steady trade winds much of the year (Douillet 1998). In a larval dispersal modeling study in the SWL, Cuif et al. (2014) showed that this weather regime was unfavorable to larval retention at lagoon and reef scales by flushing larvae in the open ocean through passes, but that certain episodic weather conditions (weaker winds that are more variable in direction) create favorable hydrodynamic conditions that allowed larvae to settle back to their natal reef. Cohesive dispersal, i.e., larvae originating from the same spawning event spending their entire pelagic larval life together and settling collectively, may also play a role in observed recruitment variability. Buston et al. (2009) suggested cohesive dispersal for *D. aruanus* larvae. We did not analyze kinship among the self-recruits found in March 2012, but birth and settlement dates for all self-recruits were estimated and two groups of settlers that were potentially born the same day and settled together were identified (table 5.6), suggesting that large peaks in self-recruitment may have been due to cohesive dispersal. Maternal size effects on phenotypic traits of larvae have been suggested to influence larval dispersal pattern with larger females contributing disproportionally to self-recruitment through larger body size at hatching or faster growth and/or earlier competency, allowing larvae to come back to their natal reef (Beldade et al. 2012). In our study, small females injected at the beginning of the reproductive season in November 2011 likely grew to become larger females in February 2012 (Table S5) potentially producing larvae best able to settle back to their natal reef in March 2012 than at the beginning of the reproductive season. We did not find differences in hatching size (otolith growth back-calculation, results not shown), but the mean PLD of larvae that settled in March 2012 was significantly shorter than the PLD of larvae of the other monthly cohorts. However, the difference in PLD between March 2012 and the other months remains small (between 2 to 3 days), so that it is unlikely that a slightly shortened PLD alone can explain the higher self-recruitment rate observed in March 2012. Favorable hydrodynamic events (Cuif et al. 2014), associated with cohesive dispersal (Buston et al. 2009), larval preference for natal habitat (Gerlach et al. 2007) coupled to strong oriented swimming capabilities (Leis et al. 2011), and maternal effects on larval life traits enhancing the capacity of larvae to come back to their natal reef (Beldade et al. 2012) may have all facilitated self-recruitment in March 2012.

Self-recruitment informs population openness (Hixon et al. 2002). Demographically open populations are dependent upon larval connectivity as opposed to closed populations that are replenished by self-recruitment (Pinsky et al. 2012). Most coral reef fish populations exist in a continuum between open and closed states (Jones et al. 2009). In this study, relatively low self-recruitment rates indicate that *D. aruanus* population openness in the SWL of New Caledonia is high at the reproductive season scale. Given that *D. aruanus* life history traits are closer to the majority of coral reef fishes, we believe that our results are relevant for other similar species in the SWL and in other comparable systems. Furthermore we showed through sampling of multiple cohorts that population openness is highly variable on a monthly scale. The relationship between openness and self-recruitment is clear (Pinsky et al. 2012) but the relationship between these metrics and population persistence is not. As underlined by Burgess et al. (2014), the total number of self-recruits is more directly related to self-persistence of local populations than self-recruitment is. In our study, differences in estimated self-recruitment between February and March 2012 (resp. 9% and 68%) were in fact compensated by differences in overall recruitment between these two months (resp. 3,454 and 305 recruits) resulting in a similar total number of self-recruits (resp. 310 and 207). Recruitment variability can result in strong variability in self-recruitment and small or undetectable self-recruitment does not mean that there are not many self-recruiting larvae arriving on their natal reef. Because recruitment is notoriously highly variable in both space and time, self-recruitment is also expected to be highly variable simply due to a "dilution" effect. This dilution effect must necessarily be smaller in isolated areas which are far away from other sources of larvae. Consequently, it is potentially not surprising to observe strong and steady self-recruitment in remote places (Planes et al. 2009) as opposed to variable and lower self-recruitment in continuous seascapes (Hogan et al. 2012). Our observations of strong variability in self-recruitment rates are consistent with local reef fish populations in a continental island such as New Caledonia being very open, receiving many self-recruits each month that are diluted in many more allo-recruits coming from neighboring reefs. This has important implications for management as open local populations with small or undetectable self-recruitment may still receive a sufficient number of self-recruits to benefit from local protection measures and ensure their persistence.

5.5 Acknowledgments

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5.6 Supplementary material

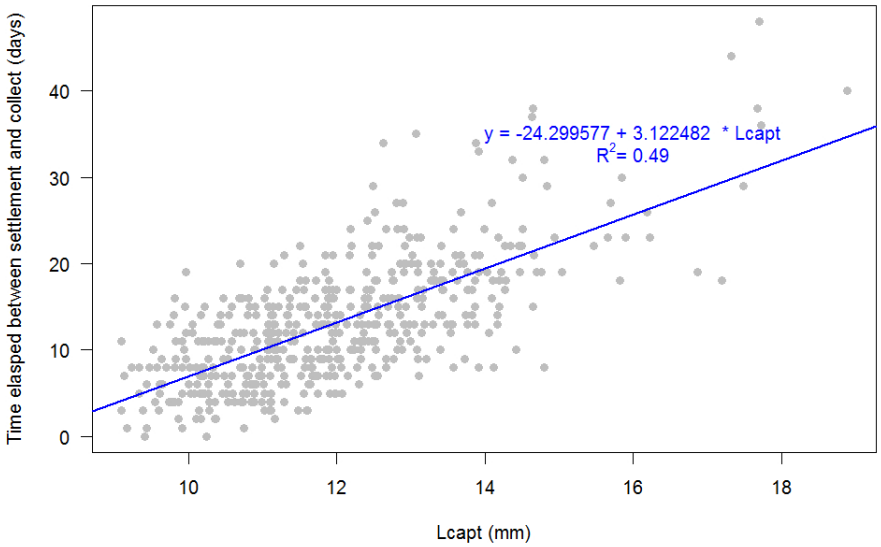

FIGURE 5.4 Time elapsed between settlement and collect (in days) in function of size at capture (Lcapt in mm) for 502 *Dascyllus aruanus* fish collected on the focal reef for which settlement date was estimated through otolith reading. Blue line represents best fit linear model.

TABLE 5.4 Settlement estimation dates of *Dascyllus aruanus* on the focal reef, number of colonies sampled, number of settlers per colony and estimated total number of settlers for each sampling month on the focal reef.

	Month	Settlement estimation dates	Number of colonies sampled	Mean nb of settlers (< 15 mm TL) per colony (± SD)	Estimated number of settlers in the focal population [95% confidence interval]
Year 1	January 2012	19 to 27 Jan. 2012	198	1.1 (± 1.9)	559 [424-693]
	February 2012	13 to 21 Feb. 2012	259	6.8 (± 6.9)	3,454 [3,028-3,881]
	March 2012	12 & 22 Mar. 2012	191	0.6 (± 1.0)	305 [233-377]
Year 2	February 2013	6 & 8 Feb. 2013	112	3.3 (± 3.8)	1,676 [1,319-2,034]
	March 2013	22 Mar. 2013	132	1.7 (± 2.4)	864 [656-1,072]

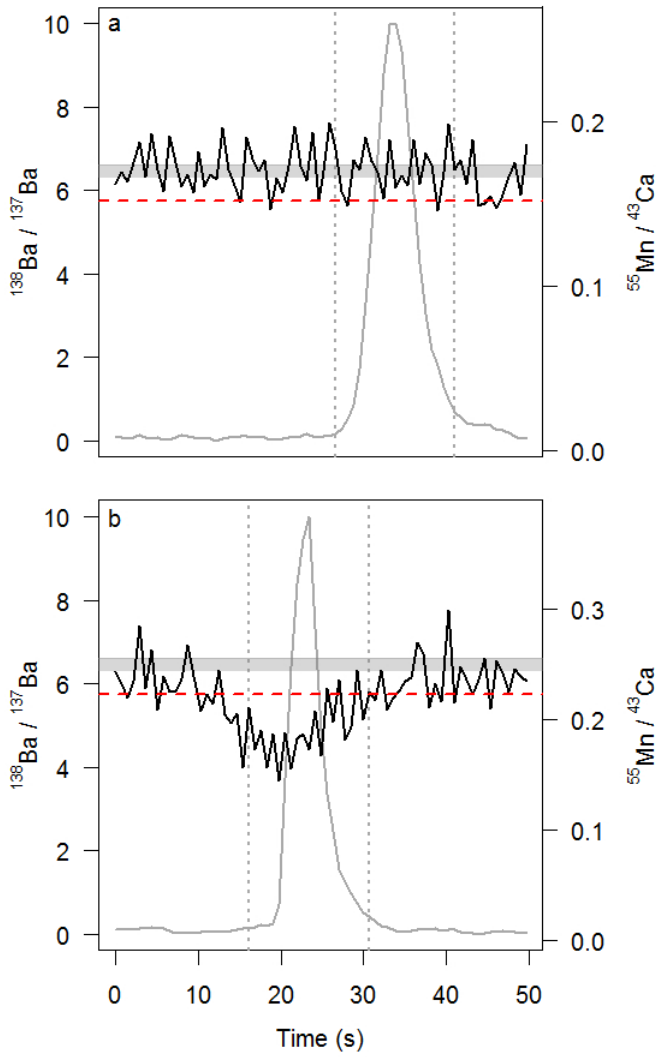

Figure 5.5 Profiles of ^{138}Ba/^{137}Ba ratio (black solid line) and ^{55}Mn/^{43}Ca ratio (grey solid line) in otoliths of a *Dascyllus aruanus* control settler (a) and a marked settler (b). X-axis represents time (seconds) elapsed since the beginning of otolith laser ablation. Grey vertical dashed lines indicate limits of otolith core. The red horizontal dashed line indicates the threshold value of 5.76. Shaded box indicates mean $\pm$ SD of the ^{138}Ba/^{137}Ba ratio in the control otoliths (n = 64).

TABLE 5.5 Operating conditions of the New Wave Research 213 nm UV laser and Agilent 7500cs inductively coupled plasma mass spectrometer (ICP-MS) used for the otoliths analysis.

Laser parameters	
Wavelength	213 nm
Frequency	5 Hz
Laser power	90%
Beam density	7 - 11 J cm^{-2}
Carrier gas	Ar 1.01 L min^{-1}
Ablation mode	Spot or scan
Ablation rate	scan : 1 µm s^{-1}
Beam diameter	30 µm
Single line scan	60 µm
ICP-MS parameters	
Optional gas	He (110 or 140)
Dwell times	^{137}Ba & ^{138}Ba (200 ms) ; ^{55}Mn (100 ms) ; ^{43}Ca (10 ms)

TABLE 5.6 Details of the 13 *Dascyllus aruanus* settlers identified as marked ordered from the lowest to the highest mean $^{138}Ba/^{137}Ba$ ratio in otolith core. "na" indicates that otolith was not read. "*" indicates that settlement date and month were estimated. The two groups of settlers that were potentially born the same day and settled together are indicated in the column "Batch event". Lcapt is total length at capture (in mm).

settler n°	$^{138}Ba/^{137}Ba$ ratio in otolith core	Lcapt (mm)	Age (days)	PLD (days)	Birth date	Batch event	Settlement date	Settlement month
657	4.06	10.5	35	21	16/02/2012	2	08/03/2012	M12
644	4.58	11.1	35	21	16/02/2012	2	08/03/2012	M12
653	4.69	10.6	27	24	24/02/2012		19/03/2012	M12
616	4.75	13.7	na	na	na		03/03/2012*	M12*
632	4.76	17.0	58	24	24/01/2012		17/02/2012	F12
669	4.90	12.6	44	22	07/02/2012	1	29/02/2012	M12
660	4.97	12.8	44	19	07/02/2012	1	26/02/2012	M12
681	5.44	12.0	na	na	na	2	08/03/2012*	M12*
637	5.51	12.6	36	20	15/02/2012	2	06/03/2012	M12
615	5.54	14.6	54	16	28/01/2012		13/02/2012	F12
640	5.61	11.5	44	22	07/02/2012	1	29/02/2012	M12
651	5.70	12.7	na	na	na	2	06/03/2012*	M12*
690	5.74	14.1	46	19	05/02/2012	1	24/02/2012	M12

Table 5.7 P.values of t.tests between mean *Dascyllus aruanus* PLDs. Significant p.values (< 0.05) are indicated in bold.

	J12	F12	M12	F13	M13
J12	1	-	-	-	-
F12	**0.0080**	1	-	-	-
M12	**2.4010E-10**	**3.8040E-03**	1	-	-
F13	0.0670	0.1497	**5.8950E-09**	1	-
M13	**0.0269**	0.5316	**8.8640E-05**	0.4163	1

Table 5.8 Number and percent of *Dascyllus aruanus* individuals measured each month on the injected colonies per size classes of total length (TL in mm). Individuals < 30 mm TL were measured but not injected.

	TL (mm)				
Injection month	< 30	[30-40[	[40-50[	[50-60[	> 60
November 2011	306 (19%)	371 (23%)	378 (24%)	363 (23%)	190 (12%)
December 2011	289 (21%)	220 (16%)	378 (27%)	347 (25%)	156 (11%)
January 2012	190 (12%)	332 (20%)	491 (30%)	416 (25%)	206 (13%)
February 2012	69 (5%)	284 (19%)	470 (31%)	467 (31%)	220 (15%)
December 2012	65 (4%)	311 (19%)	509 (32%)	534 (33%)	191 (12%)
January 2013	32 (2%)	262 (18%)	451 (31%)	487 (33%)	228 (16%)

Chapitre 6

Comparison between self-recruitment and connectivity estimates from a larval dispersal model and field observations

6.1 Introduction

Though both empirical and simulation approaches have been used to investigate marine larval retention and dispersal patterns (e.g., Saenz-Agudelo et al. (2012), Hogan et al. (2012), Treml et al. (2012), Paris et al. (2013)), results from these two complementary approaches have rarely been compared to each other in the context of a single marine system (but see Simpson et al. (2014)). In chapter 3, simulations were used to calculate larval retention rates at lagoon and reef spatial scales. Results indicate that *Dascyllus aruanus* larval retention is *a priori* possible and highly temporally variable at both spatial scales in the South-West Lagoon of New Caledonia (SWL). Similarly, in our *in situ* observations (chapter 5) we identified self-recruits and showed monthly and inter-annual variability in self-recruitment. However, these two results cannot be directly compared, for two main reasons. First, hydrodynamic forcing of the dispersal model in chapter 3 corresponds to the reproductive season 2003-2004, whereas our *in situ* observations were performed during the 2011-2012 and 2012-2013 reproductive seasons. Second, and more fundamentally, self-recruitment was estimated in the field, whereas local retention was estimated in the model. Local retention is defined as the ratio of the number of larvae that settle back to their natal population to the total number of larvae released there (Botsford et al. 2009). As such, local retention can be calculated using simulations based solely on larvae released at a single site, but is relatively inaccessible empirically because it requires estimating total larval production at a site. Self-recruitment, on the other hand, is the ratio of the number of larvae that settle back to their natal population to the total number of larvae that settle there (Botsford et al. 2009). It can be calculated empirically based solely on recruits collected at a single site. As such, it is the most commonly reported descriptor of larval dispersal in field studies (Burgess et al. 2014). Nevertheless, theoretical calculation of self-recruitment from a larval dispersal model requires releasing larvae from all sites potentially connected to the focal site, which in turn necessitates some observation (or theory-based assumption) of relative rates of larval production between sites. The objective of this chapter is to remove these barriers to direct comparison of simulated and observed patterns of larval dispersal by estimating total recruitment and self-recruitment on a central patch reef (referred to as the "focal reef" hereafter), as well as from this focal reef to ten neighboring reefs (fig. 6.1), for *D. aruanus* in the SWL using both empirical and simulation approaches.

Because the version of the hydrodynamic model MARS3D used in this chapter is slightly different from the earlier version used in chapter 3, we begin this chapter with an estimation of the consequences that this upgrade may have on the conclusions drawn in chapter 3 by comparing results obtained with both versions of MARS3D for the reproductive season extending from October 2003 to March 2004. In addition,

as the results of chapter 3 suggested El Niño (La Niña) years could lead to lower (higher) larval retention than neutral years, we attempt to verify this statement by modelling larval retention for an El Niño (October 2009 to March 2010) and a La Niña (October 2011 to March 2012) austral summer. Finally, we compare simulated and observed total recruitment and self-recruitment estimates on the focal reef, and the contribution of this reef to ten neighboring reefs in the SWL, for the 2011-2012 reproductive season. We try to bring together simulated and observed estimates by focusing first on average values of self-recruitment and connectivity and then on temporal variability of self-recruitment and total recruitment on the focal reef over the reproductive season.

6.2 Methods

6.2.1 Biophysical model

The larval dispersal model used here is very similar to the one used in chapter 3. The main difference with chapter 3 is that the hydrodynamic forcing of the individual-based model comes from an enhanced version of MARS3D (v8.19) with an improved configuration on the SWL[a]. Furthermore, we added a reef (Ilôt Sable) located approximately 8 km North of the focal reef in the habitat layer (fig. 6.1). This reef was missing in the atlas of Andréfouët and Torres-Pulliza (2004) on which we based the habitat selection in chapter 3. Horizontal and vertical resolution, MARS3D outputs record frequency, and Ichthyop calculation time step remained unchanged (report to chapter 3 for further details on these parameters).

6.2.2 Comparison between years

For comparison with chapter 3 and between reproductive seasons, we ran exactly the same simulations but with realistic hydrodynamic forcing corresponding to austral summers 2003-2004, (same as chapter 3), 2009-2010 (El Niño) and 2011-2012 (La Niña). Year selection was based on the Oceanic Niño Index published by the Climate Prediction Center of the National Oceanic and Atmospheric Administration (NOAA) National Weather Service. Release zone, settlement habitat, hatching events frequency, planktonic larval duration (PLD), settlement habitat detection distance and hypotheses about natal homing were kept the same to allow for direct comparison and are summarized in table 6.1. Precompetency duration (PC) was set at 11 days for this comparison between years. Natal lagoon retention (NLR) and natal reef retention

[a]Major changes are : (1) Delimitation of study area : slightly increased behind the barrier reef towards open ocean, (2) Open Boundary Conditions : BRAN version changed, (3) Boundary Conditions of Atmospheric forcing : WRF was forced with NCEP data instead of AMIP data, (4) Better resolution of sigma levels.

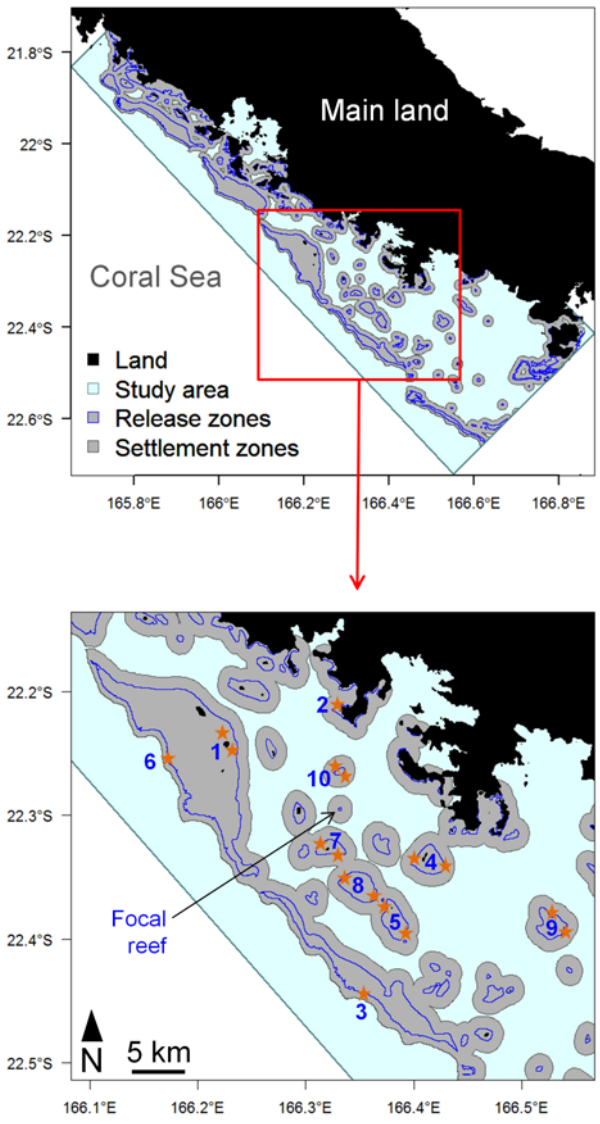

Figure 6.1 Study area : the South-West Lagoon of New Caledonia (SWL). The area in light blue represents the delimitation of the biophysical model. Release zones are delimited in blue. Settlement zones are depicted in grey and correspond to release zones buffered by a 1-km sensory zone. Land is shown in black. Localization of the focal reef and the 10 neighboring reefs (numbered from 1 to 10) is shown with orange stars indicating juveniles sampling stations on the 10 neighboring reefs.

(NRR) were calculated as the fraction of larvae released at the focal site that recruit to the lagoon and to the focal reef under both homing hypotheses, as in chapter 3. Cross-correlations between wind along-shelf and cross-shelf component and retention time series were performed as in chapter 3. Mean dispersal distances from focal reef were also calculated over each austral summer.

Table 6.1 Details of the dispersal model runs used in this chapter. PC = precompetency period. PLD = planktonic larval duration.

Run	MARS3D version	Wind forcing period	Nb releases	Nb larvae per release	Release zone	Settle zone(s)	Homing hypothesis	PC duration	Detection distance	Simulation duration (PLD)
1	old	1 Oct. 2003 to 1 Mar. 2004	1,217	500	Focal reef	All habitat	No homing	11 days	1 km	30 days
2	old	1 Oct. 2003 to 1 Mar. 2004	1,217	500	Focal reef	All habitat	Strict homing	11 days	1 km	30 days
3	new	1 Oct. 2003 to 1 Mar. 2004	1,217	500	Focal reef	All habitat	No homing	11 days	1 km	30 days
4	new	1 Oct. 2003 to 1 Mar. 2004	1,217	500	Focal reef	Focal reef	Strict homing	11 days	1 km	30 days
5	new	1 Oct. 2009 to 1 Mar. 2010	1,209	500	Focal reef	All habitat	No homing	11 days	1 km	30 days
6	new	1 Oct. 2009 to 1 Mar. 2010	1,209	500	Focal reef	Focal reef	Strict homing	11 days	1 km	30 days
7	new	1 Oct. 2011 to 1 Mar. 2012	1,217	500	Focal reef	All habitat	No homing	11 days	1 km	30 days
8	new	1 Oct. 2011 to 30 Jun. 2012	2,192	500	Focal reef	Focal reef	Strict homing	11 days	1 km	30 days
9	new	1 Oct. 2011 to 30 Jun. 2012	2,192	10,000	All habitat	All habitat	No homing	11 days	1 km	30 days
10	new	1 Oct. 2011 to 30 Jun. 2012	2,192	500	Focal reef	All habitat	No homing	21 days	1 km	30 days
11	new	1 Oct. 2011 to 30 Jun. 2012	2,192	10,000	All habitat	All habitat	No homing	21 days	1 km	30 days

6.2.3 Self-recruitment, total recruitment and connectivity

Simulations

To simulate self-recruitment on the focal reef over the 2011-2012 reproductive season, larvae were released uniformly over all suitable habitat (240 release zones in total, including the focal reef) every 3 hour from 1 October 2011 to 30 June 2012 (for PC = 11 and PC = 21 days, runs 9 and 11, respectively ; table 6.1 ; fig. 6.1). This release strategy implicitly assumes that all suitable habitat has equal reproductive output. Released larvae were randomly distributed throughout the water column from 0 to 20 m depth. As in chapter 3, we supposed that larvae became active after a given precompetency period with sensory and swimming capabilities that allowed them to detect and approach a settlement area. This non-explicit swimming behavior was included by assuming that competent larvae could actively settle once at a distance of 1 km from a settlement reef. Recruitment zones were thus defined in the model as release zones with a buffer of 1 km that lead to the definition of 27 recruitment zones including the focal reef (fig. 6.1).

Limited storage space and run time constrained us to release 10,000 larvae at each release event. As the focal reef only represents a small percentage ($\sim$ 0.03%) of the total habitat area, the number of larvae released in the focal reef was thus very small ($\sim$ 3 on average), leading to inaccurate estimates of self-recruitment. Therefore, results from both focal-reef-only and all-habitat runs were combined (i.e., runs 7 and 9 for PC = 11 days, and runs 10 and 11 for PC = 21 days) when estimating self-recruitment on the focal reef (SR) and the percentage of recruitment to other reefs (indexed i) that originated at the focal reef ($C_{i,FR}$) for each release event.

Calculations

After adjusting for the inhomogeneous number of larvae released at the focal reef in the two combined runs (i.e., $\sim$ 3 for run 9 *vs.* 500 for run 7), $C_{i,FR}$ was calculated as the ratio between recruits produced at the focal reef that recruited on reef i ($R_{i,FR}$) and recruits from all sites ($R_{i,tot}$). Numerically, this is :

$$C_{i,FR} = \frac{R_{i,FR}}{R_{i,tot}} * 100 \tag{6.1}$$

Note that SR is equal to $C_{i,FR}$ with i being the focal reef. These calculations were repeated for each spawning event along the simulated reproductive season.

Comparison with a well-mixed distribution

We compared the time-averaged $C_{i,FR}$ values to the percentage that larvae exported from the focal reef would represent among total settlement on each settlement reef i assuming a well-mixed distribution of larvae over all reefs. We hypothesized that all reefs contribute, proportionally to the release habitat (in volume) that they represent, to a randomly mixed pool of larvae that uniformly supplies reefs with recruits. Under this hypothesis, $C_{i,FR}$ should be constant over space and equal to the percentage of the total habitat that the focal reef represents. This fraction was calculated from run 9 simply as the ratio between total number of larvae released at the focal reef over the 2,192 release events (6,501) and total number of larvae released in the SWL (10,000 * 2,192).

Comparison between average simulated and field estimated self-recruitment and connectivity

We compared average (along the period December 2011 to March 2012) simulated and field estimates of self-recruitment SR and connectivity to other reefs $C_{i,FR}$ under different assumptions.

First, we hypothesized that for each release event, a fraction X of larvae released at a given site was guaranteed to recruit back to that site, independently of model results (e.g., for each release events, if X = 0.2 then 20% of larvae released in the focal reef recruited on the focal reef independently of the model results), whereas the remaining fraction 1 - X of larvae recruited according to model results. This implicitly assumes that a fraction X of larvae does not experience larval loss due to transport to unfavorable habitat.

As in section 6.2.3, the percentage $C_{i,FR}$ was calculated as :

$$C_{i,FR} = \frac{R_{i,FR}}{R_{i,tot}} * 100 \tag{6.2}$$

but with the number of recruits produced at the focal reef that recruited on reef i being :

$$R_{i,FR} = (1 - X) * R_{i,FR\,model} \tag{6.3}$$

and the total recruitment on reef i being :

$$R_{i,tot} = (1 - X) * R_{i,tot\,model} + X * S_i \tag{6.4}$$

$R_{i,FR\,model}$ and $R_{i,tot\,model}$ are the quantities given by the model and S_i is the mean number of larvae released at reef i in the model.

As before, SR is equal do $C_{i,FR}$ with i being the focal reef : in that case, R_{FR}, the number of self-recruits on the focal reef, is equal to :

$$R_{FR} = (1 - X) * R_{FR\,model} + X * S_{FR} \tag{6.5}$$

When X = 0, SR and C are as given by the model. When X = 1, all recruits are self-recruits on all sites, and none are allo-recruits.

Secondly, we assumed that the production on the focal reef was larger than in the other reefs by multiplying the number of larvae produced by this reef by $P > 1$. This effect was simply included by multiplying the number of recruits originated from the focal reef by P :

$$R_{i,FR} = P * R_{i,FR\,model} \tag{6.6}$$

$$R_{i,tot} = R_{i,tot\,model} + (P - 1) * R_{i,FR\,model} \tag{6.7}$$

Note that these quantities, and therefore SR and C, are as given by the model when P = 1.

Finally, we combined the effects represented by X and P.

Comparison between simulated and observed time series of self-recruitment and total recruitment

Putative birth dates of self-recruits estimated from the field were positioned along the simulated time series of self-recruitment for the 3-month period extending from December 2011 to February 2012. Monthly total recruitment values estimated from the field at the focal reef were also compared to simulated time series of total recruitment for the same period.

6.3 Results

6.3.1 Comparison between MARS3D versions

The slightly different versions of MARS3D used in this chapter and chapter 3 lead to similar results, with slightly smaller average dispersal distances and retention values with the new version (table 6.2).

With the new version of MARS3D, time series of NRR under both homing hypotheses show significant negative cross-correlations with the along-shore component of

Table 6.2 Comparison of retention values and dispersal distances between "old" (chapter 3) and "new" (this chapter) versions of MARS3D. NRR = natal reef retention ; NLR = natal lagoon retention ; SD = standard deviation ; SE = standard error. Run number refers to table 6.1.

		No homing			Strict homing
MARS3D version		Dispersal distance (km)	NRR (%)	NLR (%)	NRR (%)
Old	run :	1	1	1	2
	mean :	25.2	1.4	56.7	6.7
	SD :	18.1	2.3	26.4	8.2
	SE :	0.52	0.07	0.76	0.24
	range :	[0.02 ; 89.0]	[0 ; 23]	[0 ; 100]	[0 ; 42.6]
New	run :	3	3	3	4
	mean :	19.7	1.1	47.0	6.1
	SD :	14.7	1.6	20.9	6.0
	SE :	0.42	0.05	0.60	0.17
	range :	[0.04 ; 89.8]	[0 ; 14]	[7.6 ; 99.4]	[0 ; 31]

wind, as with the old version. Significant correlations occur between days 3 and 6 and between days 0 and 11 for no-homing and strict-homing hypotheses, respectively, with absolute maximum correlation coefficient of 0.3 and 0.4 occurring at a lag of 4 and 5 days, respectively (not shown). The time series of NLR shows significant negative cross-correlations with the along-shore component of wind between days 1 and 9 after release, with an absolute maximum correlation coefficient of 0.4 (not shown). These results are very similar to results of chapter 3.

With the new version of MARS3D, time series of NRR under both homing hypotheses and time series of NLR also showed significant positive cross-correlations with the cross-shore component of wind with maximum correlation coefficient of 0.2 between days 7 and 16 (NRR no homing), days 6 and 17 (NRR strict homing) and days 8 and 20 (NLR) after release.

6.3.2 Comparison between reproductive seasons

Using the new version of MARS3D, we found similar average dispersal distances and retention values across years (table 6.3). The lowest average retention is obtained for the El Niño reproductive season under both homing hypotheses (table 6.3). Regarding simulated time series, retention values are highly variable within each reproductive season for all years, without apparent synchronicity among years (fig. 6.2).

TABLE 6.3 Same as table 6.2 but using MAR3D new version for different reproductive seasons.

		No homing			Strict homing
Reproductive season		Dispersal distance (km)	NRR (%)	NLR (%)	NRR (%)
2003-2004	run :	3	3	3	4
(Neutral)	mean :	19.7	1.1	47.0	6.1
	SD :	14.7	1.6	20.9	6.0
	SE :	0.42	0.05	0.60	0.17
	range :	[0.04 ; 89.8]	[0 ; 14]	[7.6 ; 99.4]	[0 ; 31]
2009-2010	run :	5	5	5	6
(El Niño)	mean :	20.0	0.6	38.1	3.4
	SD :	15.1	1.0	18.0	4.5
	SE :	0.43	0.03	0.52	0.13
	range :	[0.09 ; 89.7]	[0 ; 6.6]	[4.4 ; 97.6]	[0 ; 26.8]
2011-2012	run :	7	7	7	8
(La Niña)	mean :	20.3	0.7	44.0	5.3
	SD :	14.4	1.1	24.9	5.9
	SE :	0.41	0.03	0.71	0.17
	range :	[0.02 ; 89.8]	[0 ; 7.2]	[2.6 ; 100]	[0 ; 27.4]

For reproductive season 2011-2012, corresponding to the field season, time series of NRR under both homing hypotheses show significant negative cross-correlations with the along-shore component of wind (fig. 6.3a). Significant correlations occur between days 6 and 11 and between days 5 and 11 for no-homing and strict-homing hypotheses, respectively, with absolute maximum correlation coefficient of 0.4 occurring at a lag of 9 days. The time series of NLR shows significant negative cross-correlations with the along-shore component of wind between days 1 and 10 after release, with an absolute maximum correlation coefficient of 0.5. These results are very similar to results obtained for the reproductive season 2003-2004 with the new and old versions of MARS3D. Time series of NLR also showed significant cross-correlations with the cross-shore component of wind with maximum correlation coefficient of 0.2 between days 6 and 8 (fig. 6.3b).

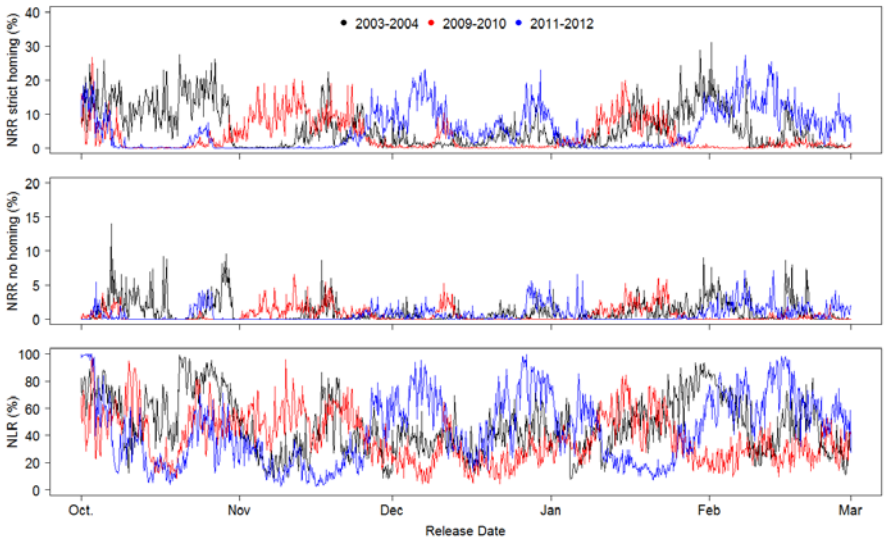

Figure 6.2 Retention time series for release dates comprised between 1st of October and 1st of March and 3 different reproductive seasons. NRR = natal reef retention ; NLR = natal lagoon retention.

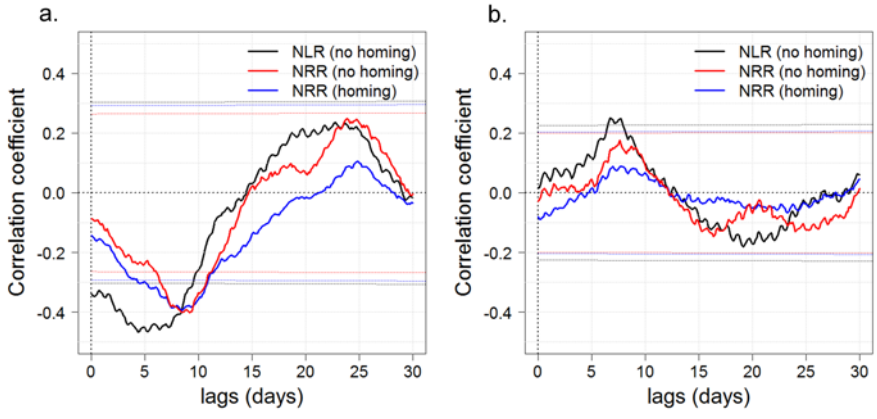

Figure 6.3 Cross-correlations between 2011-2012 retention time series and along-shore (a) and cross-shore (b) wind components derived from WRF model for a precompetency period of 11 days and under the hypotheses of strict or no homing. NLR = natal lagoon retention ; NRR = natal reef retention. Dotted lines represent cross-correlation critical values with a 95% level of confidence, adjusted to take into account temporal autocorrelation of time series.

6.3.3 Self-recruitment and total recruitment at the focal reef, and connectivity to the other reefs

Comparison between model estimates and a well-mixed distribution

Assuming a well-mixed distribution of larvae over all reefs, the percentage that larvae export from the focal reef would represent among total settlement on any settlement reef is equal to 0.03%. Simulated self-recruitment and connectivity estimates are only slightly superior to this value (table 6.4), suggesting that distribution of larvae is close to being well-mixed in the model.

Comparison between average simulated and field estimates

The average simulated value of self-recruitment SR for larvae released at the focal reef between December 2011 and February 2012 is 0.1% $\pm$ 0.1 (range = [0 ; 1.2%]). The average value of connectivity $C_{i,FR}$ from the focal reef to the ten neighboring reefs for the same period is 0.09% (table 6.4) with little variability among reefs. Table 6.4 shows the result obtained for a precompetency period (PC) of 21 days, but a similar result is obtained for PC = 11 (section 6.8).

From chapter 5, I remind the following relevant results for comparison. The average percentage of self-recruitment measured in the field at the focal reef between January and March 2012 is 21% (with a 95% confidence interval ranging from 12 to 35%). The export of larvae from the focal reef to the ten neighboring reefs during this period is estimated to be 0% ($<$ 2% with a 95% probability). Individuals that settled between January and March 2012 were born between December 2011 and February 2012 (table 6.9).

Therefore, the average simulated self-recruitment value of larvae released at the focal reef between December 2011 and February 2012 is more than two orders of magnitude lower than the percentage measured on the field. The average value of connectivity to the ten neighboring reefs is within the confidence interval of the field estimate.

A way to reconcile the average simulated and observed values of self-recruitment is to assume that the fraction of released larvae self-recruiting independently of model results is X $\sim$ 0.35 (fig. 6.4). As a consequence, the simulated connectivity values between the focal reef and the surrounding reefs ($C_{i,FR}$) decrease slightly (table 6.5) but remain consistent with the confidence interval found in the field.

Table 6.4 Simulated percentage of larvae exported from the focal reef to each settlement reef i ($C_{i,FR}$) with i = 1 to 27. Mean, standard deviation (SD), minimum and maximum obtained for a precompetency period of 21 days, and calculated for release events between December 2011 and March 2012. Second column corresponds to reefs considered in the field study.

		Connectivity $C_{i,FR}$ (%)			
i	sampled reef n°	mean	SD	min	max
1		0.03	0.02	0	0.15
2		0.04	0.05	0	0.26
3		0.05	0.05	0	0.32
4		0.01	0.07	0	0.60
5	1, 3 & 6	0.07	0.05	0	0.38
6	2	0.11	0.12	0	0.92
7		0.02	0.10	0	0.60
8		0.13	0.16	0	1.61
9		0.23	0.58	0	5.42
10	10	0.10	0.13	0	1.21
11	4	0.08	0.09	0	0.59
12	5, 7 & 8	0.09	0.10	0	0.74
13	Focal reef	0.09	0.14	0	1.21
14		0.06	0.08	0	0.44
15		0.09	0.12	0	0.68
16		0.06	0.07	0	0.49
17	9	0.07	0.09	0	1.08
18		0.05	0.08	0	0.60
19		0.05	0.10	0	1.21
20		0.06	0.11	0	1.21
21		0.04	0.10	0	1.21
22		0.06	0.16	0	1.21
23		0.04	0.08	0	0.60
24		0.04	0.10	0	0.60
25		0.04	0.11	0	1.21
26		0.03	0.12	0	0.60
27		0.10	0.14	0	1.81

Another way to reconcile the average simulated and observed values of self-recruitment is to assume that the focal reef produces P $\sim$ 450 times more larvae than the other reefs (fig. 6.5). However, as a consequence, simulated connectivity values between the focal reef and the surrounding reefs increase significantly (table 6.5) exceeding the values found in the field.

Finally, combining the effects represented by X and P with, e.g., P $\sim$ 10 and X $\sim$ 0.1, increase simulated self-recruitment SR to the observed value of 21% while keeping connectivity values at $<$ 1% consistently with observations (fig. 6.6, table 6.5).

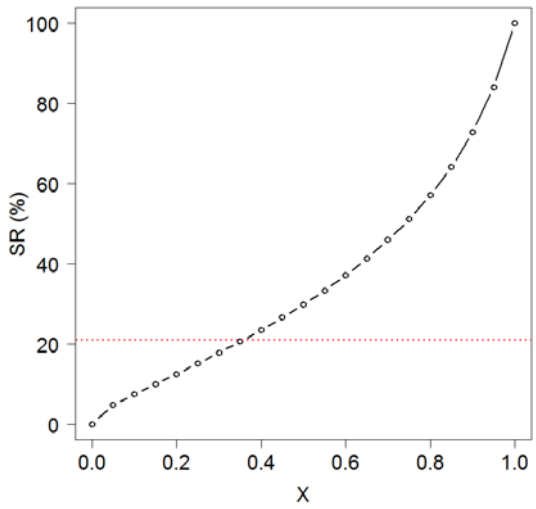

FIGURE 6.4 Average simulated value of self-recruitment at the focal reef (SR) obtained for increasing values of X. Red dotted line indicates SR measured on the field.

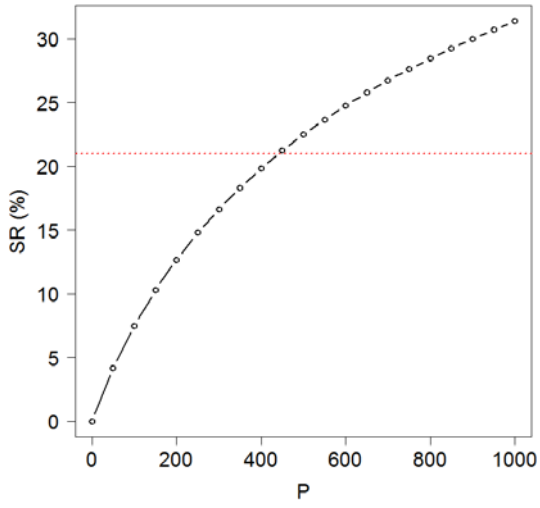

FIGURE 6.5 Same as fig. 6.4 for increasing values of P (and X = 0).

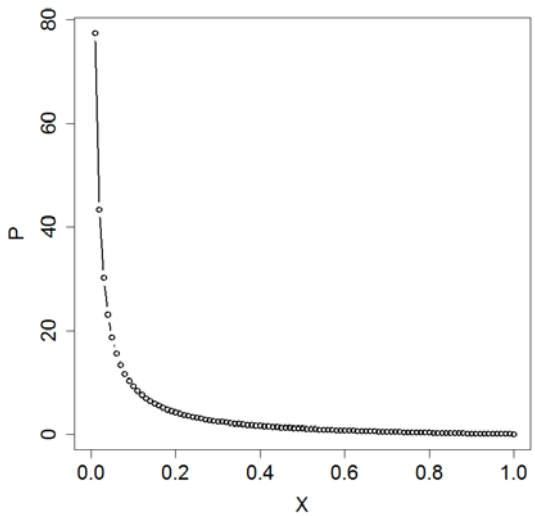

Figure 6.6 Combinations of parameters X and P that result in average simulated SR $\sim$ 21%.

TABLE 6.5 Same as table 6.4 for three different combinations of parameters X and P.

		Connectivity $C_{i,FR\,(\%)}$											
		X = 0.35 ; P = 1				X = 0 ; P = 450				X = 0.1 ; P = 10			
i	sampled reef n°	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
5	1, 3 & 6	6E-03	5E-03	0E+00	0.03	22.46	12.41	0.00	63.41	0.21	0.18	0	0.94
6	2	0.07	0.06	0	0.44	27.20	17.84	0	80.76	0.92	0.88	0	7.03
10	10	0.06	0.06	0	0.37	23.67	20.92	0	84.59	0.81	0.94	0	5.91
11	4	0.04	0.04	0	0.21	22.13	17.21	0	72.79	0.63	0.67	0	4.00
12	5, 7 & 8	0.02	0.02	0	0.13	22.97	18.89	0	76.92	0.44	0.51	0	3.68
17	9	0.01	0.02	0	0.11	18.06	18.20	0	83.15	0.37	0.45	0	3.74

Comparison between simulated and observed time series of self-recruitment and total recruitment at the focal reef

With X = 0.1 and P = 10, fig. 6.7 shows time series of self-recruitment and total recruitment simulated at the focal reef. Simulated self-recruitment is different from zero at estimated birth dates of self-recruits collected in the field (fig. 6.7). Nevertheless, the model suggests a peak of self-recruitment for larvae released in mid-January 2012 although we did not find self-recruits born at this period in the field.

Field measurement of total recruitment at the focal reef showed a peak in mid-February 2012 at 6.8 recruits per colony, corresponding to larval hatching dates between late December 2011 and late January 2012 (table 6.6), as opposed to lower recruitment (1 recruit per colony) assessed in late January 2012 and mid-March 2012, corresponding to larvae released during December 2011 and between mid-January and mid-February 2012, respectively (table 6.6). The simulation of total recruitment at the focal reef suggests an opposite temporal pattern (table 6.6).

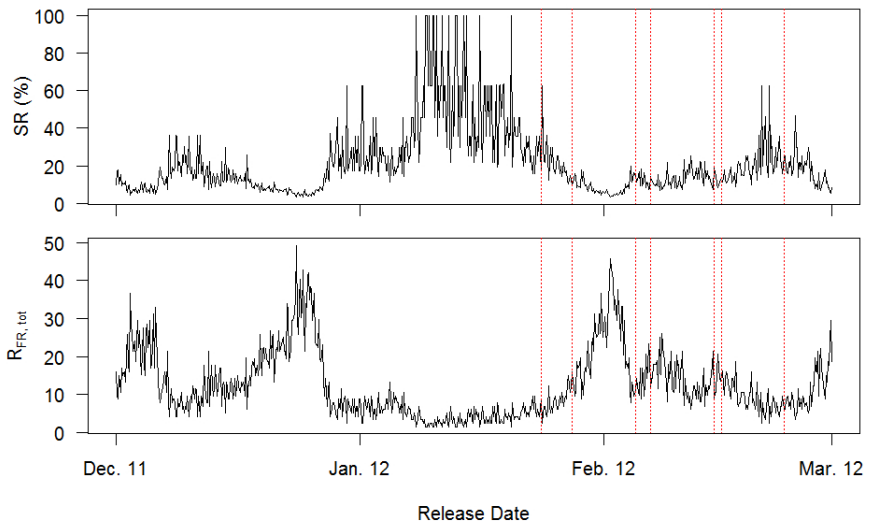

Figure 6.7 Simulated time series of self-recruitment (SR) and total recruitment ($R_{FR,tot}$) at the focal reef for larvae released between December 2011 and March 2012. Vertical red dotted lines indicate the estimated birth date of the 10 self-recruits identified in chapter 5 for which otolith was read (table 5.6, chapter 5).

TABLE 6.6 Dates of recruitment estimation surveys at the focal reef from January to March 2012. Minimum, maximum and average age of recruits (< 15 mm TL) that were counted for this estimation. Estimated birth date interval of recruits and mean number of recruits per colony estimated on the field. Mean number of recruits at the focal reef simulated with the model.

Date of survey	Age at collect (days)			Estimated birth date		Mean number of recruits per colony estimated in the field	Mean number of recruits at the focal reef simulated with the model (average $R_{FR,tot}$)
	min	max	mean	min	max		
23/01/2012	28	54	39	30/11/2011	26/12/2011	1.1	17.8
17/02/2012	25	54	37	25/12/2011	23/01/2012	6.8	7.1
13/03/2012	25	57	38	16/01/2012	17/02/2012	0.9	13.3

6.4 Discussion

Our simulated estimates of the contribution of the focal reef to the recruitment on neighboring reefs (0.09%) is consistent with the very small value derived from the field (< 2%). However, there is a large discrepancy between simulated (0.1%) and observed (21%) estimates of self-recruitment at the focal reef. Similarly, time series of simulated self-recruitment and of total recruitment at the focal reef did not match observed patterns. These discrepancies between observed and simulated results may be due to a range of biotic and abiotic factors.

Regarding the average value of self-recruitment at the focal reef, we showed that the combination of two additional factors may bring back together simulated and observed estimates. These factors were based on two assumptions, firstly that a fraction of larvae recruited to their natal reef independently of model results (thereafter referred to as "homing" factor) and secondly that the production of the focal population was higher than on the other reefs ("production" factor).

The introduction of the "homing" factor refers to a homing behavior (Gerlach et al. 2007) or a sticky water effect (Andutta et al. 2012). In chapter 3, we already introduced the notion of homing in the model by distinguishing two hypotheses : no homing and strict homing. The strict-homing hypothesis implied that larvae could only settle in their natal reef, naturally leading to 100% of self-recruitment and 0% of export from any reef to neighboring reefs, which is incompatible with our field results. In this chapter, we modelled larval dispersal under the no homing hypothesis, assuming that larvae could settle in all suitable habitat, leading to much lower values

of self-recruitment. This, again, results in simulated values of self-recruitment that are incompatible with our results. The "homing" factor used in this chapter allows modulating results according to a fraction of released larvae that are "guaranteed" to recruit to their natal reef. This factor may also be interpreted as a local circulation effect. Even if the resolution of our biophysical model is fine (horizontal resolution of 500 m and 30 terrain following generalized sigma levels in the vertical dimension), it may still not be enough to represent currents at the very small spatial scale of patch reefs and thus miss to model processes like sticky water and Stokes drift that could contribute to retention of larvae (Andutta et al. 2012, Fujimura et al. 2014).

The "production" factor allows for considering heterogeneous production over all suitable habitat used in the model. The uniform release of larvae over all suitable habitat in the Ichthyop tool indeed implicitly assumes that adult density is homogenous over all suitable habitats. But production is likely to be heterogeneous in the wild. For example, we found that *D. aruanus* adult density may vary by one order of magnitude among sampled reefs (e.g., 0.17 adults per m^2 on reef 8 *vs.* 1.23 adults per m^2 on reef 7). The "production" factor can also be interpreted as a differential mortality in favor of self-recruits (Vigliola and Meekan 2002, Meekan et al. 2006, Nanninga 2013). Selective mortality occurring in favor of larvae originating from the focal reef during dispersal or settlement may indeed influence self-recruitment significantly by increasing the proportion of self-recruits.

When used separately, these factors must be included at such high values (e.g., a production 450 times higher at the focal reef) that they are likely not compatible with the reality. When used in combination, however, the values are much lower (e.g., a production 10 times higher at the focal reef with 10% of larvae recruiting to their natal reef), although these can only be confirmed via additional field research.

Beside the "homing" and "production" factors, other factors were considered as being potentially responsible for the differences between observed and simulated results. For example, *D. aruanus* habitat was defined in the model as all reefs shallower than 20 m (Allen 1991) based on GIS habitat maps provided by the atlas of coral reefs in New Caledonia (Andréfouët and Torres-Pulliza 2004) and supported by knowledge gathered from field work (section 6.5). But habitat might be more restricted. For example, we observed that most *D. aruanus* colonies were localized between 8 and 12 meters on patch reefs in the SWL and we showed in chapter 3 that release depth may influence larval retention, with better retention when release depth was deeper. In our simulations, estimates of self-recruitment generally did increase with release depth (section 6.6) but the values obtained for the 8 to 12 m depth levels were only slightly higher than the average. We also identified a major source zone, localized on the barrier

reef North-West of the focal reef, that represents 30% of the total released larvae in the SWL that recruits on the focal reef (section 6.7). This zone is principally composed of shallow terrace (section 6.5), which is not the preferred habitat of *D. aruanus*. However, excluding this zone lead to a 30% increase in self-recruitment.The age of competence might also influence dispersal patterns by increasing/decreasing the passive dispersal phase and thus decreasing/increasing larval retention at both reef and lagoon scale (chapter 3). However, for a shorter precompetency period of 11 days we found very close estimates as with a precompetency of 21 days (section 6.8). Furthermore, setting the precompetency duration at 21 days in the model gives settlement ages that are closer to the mean PLD of 3 weeks estimated for *D. aruanus* in the SWL (chapter 2). These results suggest that these different factors are unlikely to play a major role in increasing the simulated self-recruitment value to the level required to match observations.

The comparison between temporal variability of simulated and observed self-recruitment and total recruitment at the focal reef also showed differences. When a constant "homing" factor is included, the variability in the simulated number of self-recruits is much reduced. Consequently, the temporal variability of the percentage of self-recruitment is mostly driven by, and the inverse of, the temporal variability of total recruitment in the model. But the model failed to reproduce the variability of total recruitment properly. It suggested a peak of recruitment for larvae released in December 2011 that was not observed in the field and weak recruitment for larvae released in January 2012 while observed recruitment peaked. We thought that these contrasting results could be due to our model assumption that egg production is constant during the reproductive season. As shown in chapter 2, this production might actually be variable according to the gonadosomatic index (GSI). We used GSI as a correction factor of total recruitment but this proved insufficient to change the simulated temporal variability of total recruitment significantly (section 6.9). The planktonic larval duration (PLD), and therefore the duration of the precompetency period, might also change during the reproductive season according to water temperature with longer PLD at the beginning of the reproductive season leading to weaker recruitment. Including this effect in the model might help to inverse the tendency in the simulated recruitment peaks. Finally, it may well be that the temporal variability associated to the processes represented by our factors X and P might also govern the temporal variability of observed self-recruitment and total recruitment.

Comparing simulated and observed data of larval dispersal is a complicated task as discrepancies might arise from a range of reasons that are often difficult to disentangle without additional research. Nevertheless, one of the interests of this work is to draw attention to the most plausible explanations of these differences and prioritize future

work directions. Here we showed that the habitat of *D. aruanus*, because it drives the number of larvae released, must be better known as it is likely not as homogenous, and maybe more restricted, than previously assumed. Then we showed that future work should focus on possible biotic or abiotic processes (e.g., homing behavior, local circulation patterns) that allow larvae to recruit back to their natal population much more than when these processes are not considered.

6.5 Appendix A

TABLE 6.7 Habitat selection for *Dascyllus aruanus* in the biophysical model : selected gridcodes and corresponding attributes in (Andréfouët and Torres-Pulliza 2004).

Gridcode	L2 attrib	L3 attrib	L4 attrib
658	Continental patch complex	Intra-lagoon patch-reef complex	Shallow terrace
665			Forereef
666			Reef flat
829	Continental outer shelf barrier	Outer barrier reef complex	Shallow terrace
830			Shallow terrace with constructions
834			Deep terrace with constructions
929	Continental fringing	Lagoon exposed fringing	Forereef
930			Reef flat
934			Shallow terrace
937		Diffuse fringing	Diffuse fringing

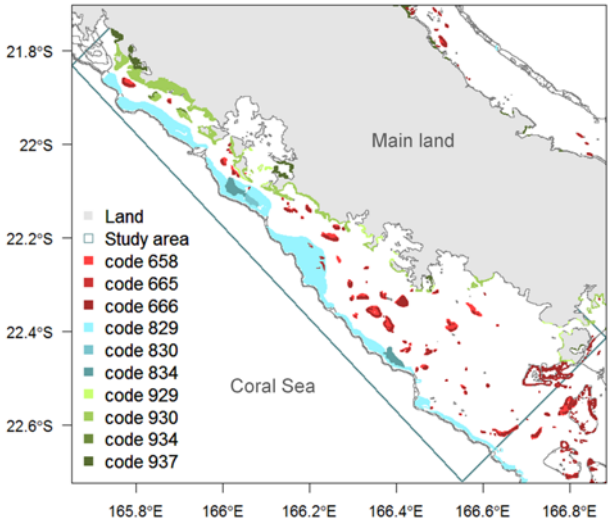

FIGURE 6.8 Habitat selection map for *Dascyllus aruanus* in the biophysical model. Barrier reef habitat is depicted in blue, patch reef habitat is in red and fringing reef habitat is in green. Codes correspond to selected gridcodes in Andréfouët and Torres-Pulliza (2004).

6.6 Appendix B

The influence of release depth on the rate of simulated self-recruitment at the focal reef was estimated. To do so, we used the same equation as insection 6.2.3 for different release depth intervals. Release depth intervals were grouped into 2 m bins beginning at 0m and going to 18m (no habitat is available at the focal reef between 18-20 m depth). Except for the release depth interval [−18 m, −16 m] for which very few larvae were released from the focal reef (12 larvae per release on average), the percentage of self-recruitment increased with increasing release depth with a maximum of 0.18% reached for the release depth interval [−16 m, −14 m]. As most of *D. aruanus* colonies were observed between 8 and 12 meters depth on patch reefs in the SWL, considering these release depths would only multiplied simulated self-recruitment by approximately 1.5 which is too low to reach the value of self-recruitment observed on the field.

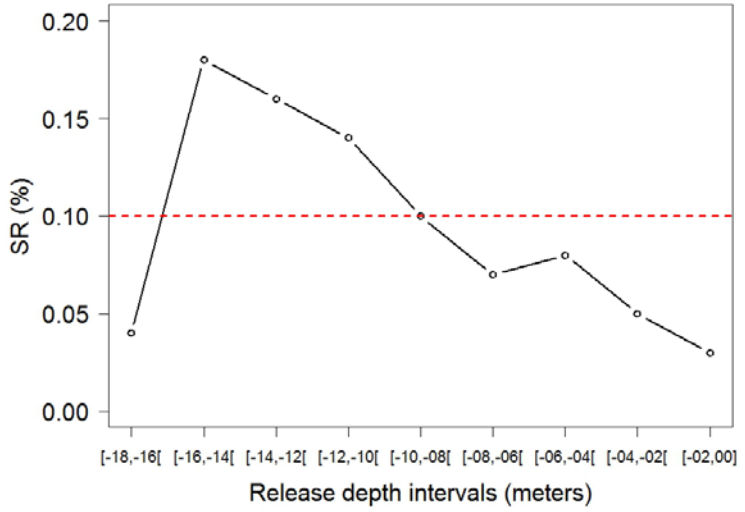

Figure 6.9 Simulated SR obtained for different release depth intervals and averaged for spawning events that occurred between December 2011 and March 2012. Red dotted line indicates averaged simulated SR for the release depth interval [-20 m, 0 m].

6.7 Appendix C

We mapped the departure localization of larvae that recruited on the focal reef to identify potential major source zones in the model, by plotting the number of larvae released in each square grid cell of 0.01° spatial resolution from December 2011 to March 2012.

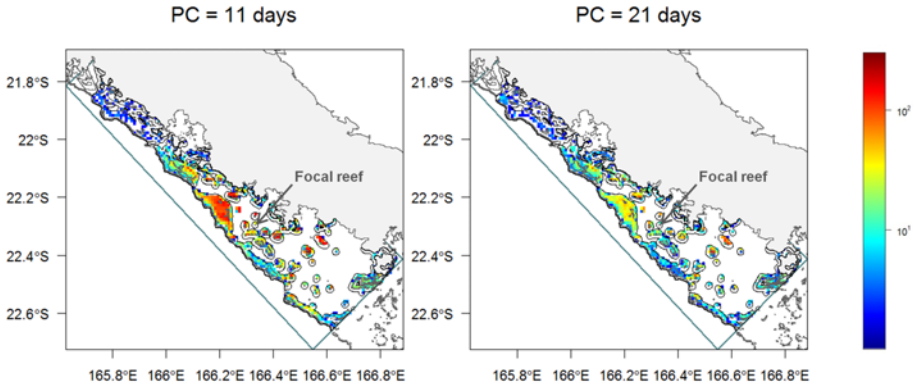

FIGURE 6.10 Number of focal reef's recruits that were released in each grid cell of 0.01° in size from December 2011 to March 2012 for both precompetency periods (PC), calculated on a logarithmic scale as $\log_{10}(N_i + 1)$ where N_i is the number of larvae release in grid cell i.

6.8 Appendix D

Table 6.8 Same as table 6.4 (main text) but for a precompetency period of 11 days.

		Connectivity $C_{i,FR}$ (%)			
i	sampled reef n°	mean	SD	min	max
1		0.02	0.02	0	0.12
2		0.03	0.05	0	0.26
3		0.03	0.03	0	0.19
4		0.00	0.03	0	0.39
5	1, 3 & 6	0.07	0.05	0	0.29
6	2	0.12	0.16	0	1.56
7		0.03	0.23	0	3.53
8		0.19	0.27	0	1.77
9		0.38	1.25	0	12.96
10	10	0.11	0.15	0	1.06
11	4	0.11	0.14	0	0.82
12	5, 7 & 8	0.07	0.08	0	0.59
13	Focal reef	0.10	0.14	0	1.18
14		0.08	0.13	0	0.82
15		0.09	0.13	0	0.72
16		0.06	0.09	0	0.66
17	9	0.05	0.08	0	0.72
18		0.03	0.07	0	0.49
19		0.03	0.05	0	0.29
20		0.03	0.06	0	0.59
21		0.02	0.06	0	0.59
22		0.03	0.10	0	1.77
23		0.02	0.05	0	0.67
24		0.02	0.08	0	1.18
25		0.03	0.08	0	0.64
26		0.03	0.10	0	1.18
27		0.11	0.16	0	1.53

6.9 Appendix E

We corrected the simulated total recruitment by multiplying it by the gonadosomatic index (GSI) derived from chapter 2. We hypothesized that GSI data were the same each year and fitted a local polynomial regression to our GSI data with the function *loess* in R (Cleveland et al. 1992) that allowed us to predict a GSI value for each release date (fig. 6.11).

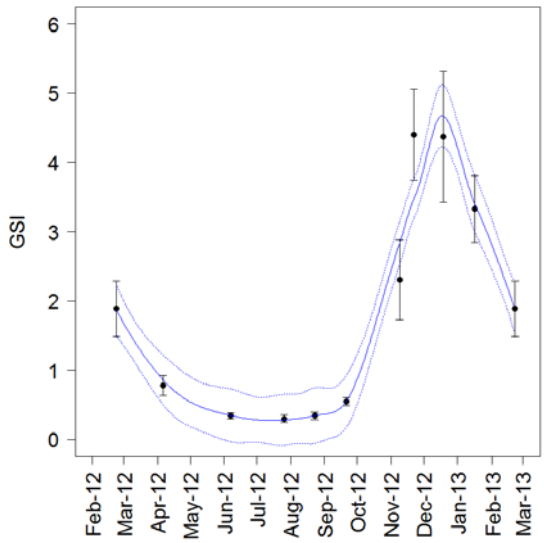

FIGURE 6.11 Mean gonadosomatic index (GSI) measured for *Dascyllus aruanus* from February 2012 to January 2013 (n = 130 fish). Error bars represents standard error (SE). Solid blue line indicates fitted local polynomial regression. Dotted blue lines indicate standard errors on predicted values.

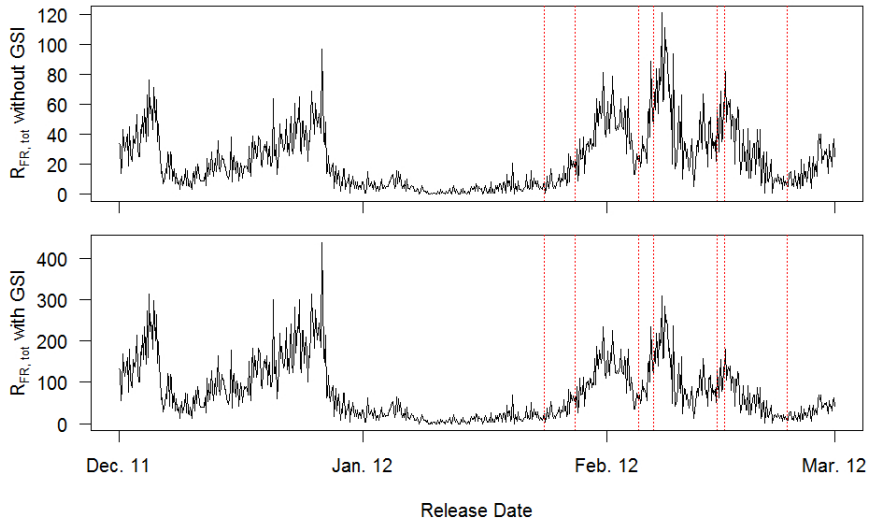

FIGURE 6.12 Simulated total recruitment time series on the focal reef ($R_{FR,tot}$) without and with GSI correction.

TABLE 6.9 Estimated birth date interval of recruits (< 15 mm TL) that were counted for the estimation of total recruitment on the focal reef in the field and mean number of recruits per colony estimated on the field. Mean number of recruits at the focal reef simulated with the model, without and with GSI correction.

Estimated birth date		Mean number of recruits per colony estimated in the field	Average $R_{FR,tot}$ without GSI	Average $R_{FR,tot}$ with GSI
min	max			
30/11/2011	26/12/2011	1.1	17.8	79.4
25/12/2011	23/01/2012	6.8	7.1	28.9
16/01/2012	17/02/2012	0.9	13.3	36.3

Chapitre 7

Conclusion

Cette thèse s'inscrit dans un contexte de demande croissante en connaissances sur la dispersion larvaire des espèces marines, connaissances qui sont indispensables à une meilleure compréhension de la structure et de la dynamique des populations et à une gestion avisée des ressources marines (Botsford et al. 2009, Berglund et al. 2012, Sale 2013). La dispersion larvaire a longtemps été considérée comme une boite noire en écologie marine due à la difficulté d'observer des individus extrêmement petits et nombreux dans l'immensité de l'océan (Sale et al. 2005). Des progrès ont été faits ces 15 dernières années avec le développement de différentes approches, par modélisation numérique ou marquages génétiques et chimiques, pour étudier la dispersion larvaire à l'échelle démographique (Jones et al. 2009, Leis et al. 2011, Kool et al. 2013). Ces techniques ont été développées de façon plus ou moins indépendantes par des communautés de chercheurs différentes et le défi actuel réside dans une confrontation de ces approches complémentaires (Jones et al. 2009, Leis et al. 2011, Kool et al. 2013). La plupart des études comparant les résultats de modèles de dispersion larvaire avec des mesures *in situ* concernent la connectivité génétique et s'appuient sur des mesures indirectes des flux de gènes entre populations (e.g., Gilg and Hilbish (2003), Galindo et al. (2006), Foster et al. (2012)). Or il existe un réel besoin de confronter les modèles de dispersion larvaire à la réalité, à une échelle démographique, afin de pouvoir les utiliser de façon opérationnelle dans des modèles de dynamique de populations (Selkoe et al. 2008, Cowen and Sponaugle 2009, Jones et al. 2009, Burgess et al. 2014).

Ce travail de comparaison entre les résultats d'un modèle de dispersion larvaire et des mesures *in situ* de l'auto-recrutement et de la connectivité démographique obtenues par marquages, est extrêmement rare. Cette rareté s'explique tout d'abord par la nouveauté et la difficulté de mettre en oeuvre des études de mesures *in situ* de la connectivité démographique, qui sont par conséquent souvent limitées à un épisode de dispersion unique (Jones et al. 1999, Almany et al. 2007, D'Aloia et al. 2013, Schunter et al. 2014), ou répliquées à l'échelle de quelques années (Jones et al. 2005, Carreras-Carbonell et al. 2007, Carson et al. 2010, Hogan et al. 2012, Saenz-Agudelo et al. 2012) mais très rarement répliquées à plus haute fréquence (Chittaro and Hogan 2013, Cook et al. 2014). Or une comparaison robuste avec des résultats de modèle nécessite d'avoir une série temporelle consistante de mesures *in situ*. D'autre part, la résolution des modèles biophysiques doit être adaptée à l'échelle d'étude de la connectivité démographique, souvent de l'ordre de quelques centaines de mètres à plusieurs dizaines de km (Jones et al. 1999, Cowen et al. 2000, Jones et al. 2005, Almany et al. 2007, Planes et al. 2009, Saenz-Agudelo et al. 2012) ce qui requiert une résolution fine des processus hydrodynamiques. Enfin la comparaison entre modèle et données nécessitent de choisir des métriques comparables. Par exemple, une des métriques les plus communément mesurée *in situ* pour mesurer le taux de retour des larves à leur

population d'origine est l'auto-recrutement (mesurée par rapport au nombre total de recrues dans cette population) alors que les modèles biophysiques fournissent la plupart du temps une mesure de la rétention locale (calculée par rapport au nombre total de larves produites par la population d'origine).

Dans ce contexte, l'objectif de ce travail de thèse était d'étudier la dispersion larvaire à une échelle de temps écologique en utilisant la modélisation biophysique et les marquages chimiques, au sein d'un même écosystème marin où aucune étude n'avait encore été menée sur la dispersion larvaire, le lagon sud-ouest de Nouvelle-Calédonie (SWL), et en s'appuyant sur un poisson de récif, la demoiselle à queue blanche (*Dascyllus aruanus*).

7.1 Principaux résultats

La synthèse bibliographique sur la biologie et l'écologie de *Dascyllus aruanus* réalisée dans le chapitre 2, et complétée par les connaissances acquises au cours de la thèse sur sa reproduction et son stade larvaire, permet de conclure que *D. aruanus* est une espèce modèle adéquate pour étudier la connectivité par dispersion larvaire au sens où elle est représentative de nombreuses autres espèces de poissons démersaux, notamment en termes de stratégie de reproduction, de durée de vie larvaire, et de domaine vital (Wellington and Victor 1989, Guillemot et al. 2011). Comme beaucoup d'espèces de poissons démersaux, cette espèce possède en effet un domaine vital extrêmement limité au stade adulte et les échanges d'individus entre populations locales ne peuvent se faire que pendant le stade larvaire pélagique. Cette espèce est facile à identifier, collecter et manipuler, et elle s'adapte bien à la captivité, ce qui facilite son étude. De plus, *D. aruanus* se reproduit avec une fréquence élevée sur une longue période de reproduction ; il est donc possible d'étudier la variabilité temporelle de la connectivité. Enfin, *D. aruanus* possède une aire de répartition très vaste à travers l'Indo-Pacifique rendant possible la mise en place d'études comparatives dans de nombreux écosystèmes.

La circulation des masses d'eau dans le SWL est soumise à des vents d'alizés pendant une majeure partie de l'année (Douillet 1998). Ce forçage atmosphérique entraîne des temps de résidence relativement courts des eaux à l'intérieur du lagon (Jouon et al. 2006, Ouillon et al. 2010), et peut donc se révéler extrêmement défavorable à la rétention des larves et donc aux connexions entre populations locales lagonaires pour des espèces dont la durée de vie larvaire excède ces temps de résidence. Le modèle biophysique de dispersion larvaire mis en place dans le chapitre 3 se base sur des champs de courants issus d'un modèle hydrodynamique forcé par des vents réalistes et prend en compte l'habitat de *D. aruanus*, sa saison de reproduction, sa

durée de vie larvaire et de pré-compétence. Ce modèle montre que la rétention larvaire est possible à l'échelle d'une population locale et à l'échelle du lagon, atteignant périodiquement des valeurs beaucoup plus grandes que prévues, et qu'elle présente une grande variabilité temporelle. Les résultats de corrélations croisées entre séries temporelles de rétention et de régimes de vent suggèrent que la rétention larvaire de *D. aruanus* au sein du lagon peut être prédite par des conditions climatiques à large échelle. Des conditions climatiques caractérisées par de forts alizés conduisent à une rétention larvaire très faible aux deux échelles spatiales considérées. En revanche, des taux de rétention élevés sont permis par des conditions climatiques caractérisées par des vents plus faibles en intensité et de direction plus variable. Les distances de dispersion sont relativement courtes, de l'ordre de 25 à 35 km selon l'hypothèse faite sur la durée de la pré-compétence, ce qui suggèrent une connectivité entre populations locales à l'intérieur du lagon.

Afin de confronter ces résultats de modèle à la réalité, une expérience de marquage chimique des larves a été menée dans le SWL (chapitre 4). Le marquage transgénérationnel est une technique récente permettant un marquage de masse *in situ* par transmission maternelle d'une marque chimique artificielle aux pièces calcifiées des embryons (Thorrold et al. 2006). L'efficacité de cette méthode a été prouvée pour plusieurs espèces de poissons et de céphalopodes (e.g., Munro et al. (2009), Pecl et al. (2010), Starrs et al. (2014)). Cependant, elle n'a été utilisée qu'une fois en milieu naturel pour estimer l'auto-recrutement (Almany et al. 2007). Avant d'utiliser cette méthode dans le milieu naturel il est primordial de tester son efficacité et ses impacts potentiels sur les adultes et les larves. C'était l'objet de l'expérience menée dans le chapitre 3 dont les résultats montrent (1) l'efficacité de la plus petite dose testée (0.5 µg de ^{137}Ba par gramme de femelle) pour marquer des larves pendant au moins 1 mois, et (2) l'absence d'effet négatif d'injections uniques ou répétées tous les mois sur le succès reproducteur, la taille des oeufs un jour après la ponte et la taille des larves âgées de deux jours. En conclusion, des injections mensuelles de solution enrichie en ^{137}Ba à 0.5 µg de ^{137}Ba par gramme de femelle permettent donc de marquer sans danger des larves de *D. aruanus* sur la durée d'une saison de reproduction. Ce chapitre développe également une nouvelle méthode d'analyse chimique des otolithes de larves âgées de deux jours par analyse au spectromètre de masse à ablation laser permettant de valider le succès de marquage tout en réduisant la durée, et donc les coûts, de l'élevage larvaire.

L'application de cette technique de marquage à une population de *D. aruanus* située au centre du SWL fait l'objet du chapitre 5. Cette population a été marquée mensuellement sur deux saisons de reproduction (étés austraux 2011-2012 et 2012-2013) afin d'estimer la variabilité temporelle de l'auto-recrutement et la contribution

de cette population au recrutement des populations de récifs voisins. L'analyse de plusieurs cohortes de jeunes recrues a permis de mettre en évidence une variabilité intra-saisonnière, mensuelle (de 0% à 68%), et une variabilité inter-annuelle (21% en 2012 et 0% en 2013) de l'auto-recrutement. L'analyse spatiale montre quant à elle une contribution nulle, ou du moins extrêmement réduite ($< 2\%$), de la population focale au recrutement des récifs voisins. Ces résultats confirment les résultats obtenus avec le modèle du chapitre 3 en montrant que le retour de larves à leur population natale est possible dans le lagon sud-ouest, suggérant l'existence de conditions climatiques et/ou de comportements larvaires favorables à la rétention. Avec une valeur moyenne d'auto-recrutement de 12% sur deux saisons de reproduction, cette population de *D. aruanus* est intermédiaire en termes d'ouverture comparée aux études conduites dans d'autres écosystèmes pour des espèces similaires (e.g., Berumen et al. (2012), Saenz-Agudelo et al. (2012)). Ce travail souligne enfin l'influence du choix du seuil utilisé pour déterminer si un individu est marqué ou non sur les valeurs estimées de l'auto-recrutement et de la connectivité.

La confrontation entre modèle et données s'appuie sur trois métriques : (1) le pourcentage d'auto-recrutement dans la population locale, mesuré par les marquages du chapitre 4 et modélisé dans le chapitre 6, plutôt que la rétention locale, utilisée dans le chapitre 3 qui informe également sur le retour des larves à leur population natale mais est beaucoup plus difficile à estimer dans le milieu naturel ; (2) la contribution de cette population locale au recrutement de 10 récifs voisins situés dans un rayon de 20 km ; (3) la variabilité temporelle du recrutement total dans cette population focale en s'appuyant sur l'été austral 2011-2012. Au niveau qualitatif, modèle et données se rejoignent sur l'importance de la variabilité temporelle de l'auto-recrutement et du recrutement total dans la population locale. Du point de vue quantitatif, modèle et données se rejoignent également sur la contribution extrêmement faible de la population focale au recrutement des récifs voisins. Le principal point de désaccord entre modèle et donnée réside dans la quantification du pourcentage d'auto-recrutement, avec un écart de plus de deux ordres de grandeur : 0.1% estimé par le modèle et 21% mesuré sur le terrain en moyenne sur la même saison de reproduction. Le chapitre 6 montre que la prise en compte de certains éléments permet une meilleure adéquation entre modèle et données, mais elle repose sur des hypothèses très fortes sur la capacité des larves à revenir à leur population natale et/ou sur l'importance de la production de la population natale par rapport aux autres populations locales lagonaires, dont la corroboration nécessitera des études qui dépassent le cadre de ce travail.

Les résultats de ce travail permettent néanmoins de conclure qu'à l'échelle du SWL (i.e., quelques dizaines de kilomètres), les populations de *D. aruanus* sont interconnectées, avec un pourcentage d'auto-recrutement très fluctuant mais pouvant être

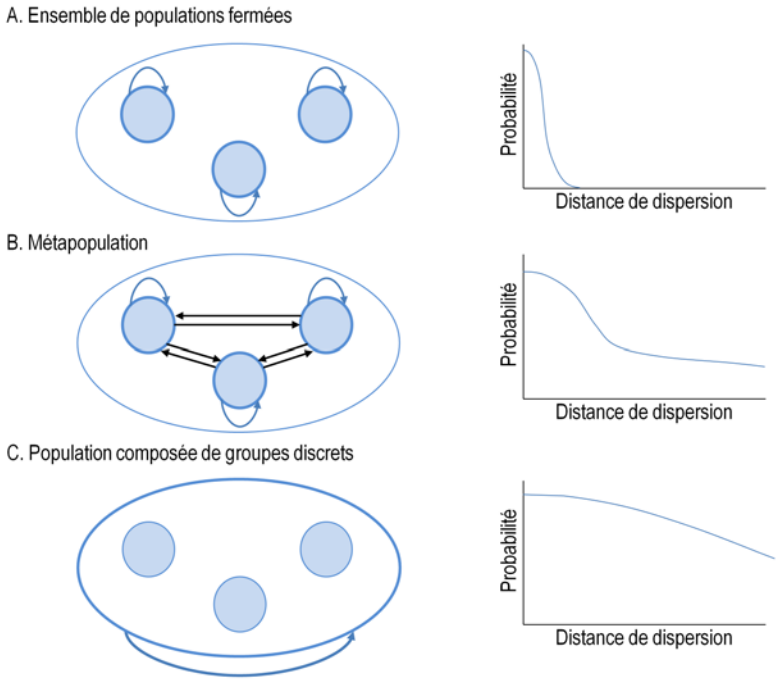

Figure 7.1 Adaptée de Kritzer and Sale (2004). Trois types de populations structurées spatialement. Les petits disques représentent des populations locales discrètes à l'intérieur d'une région plus large représentée par un ovale. Les lignes épaisses définissent l'échelle spatiale à laquelle se situe la dynamique de population. Pour chaque type de structures sont représentées les courbes de dispersion schématique de chaque population locale. (A) Populations locales fermées avec des dynamiques indépendantes sans échange d'individus à l'échelle démographique ; les distances de dispersion sont très courtes par rapport aux distances séparant les populations. (B) Métapopulation ; les distances de dispersion sont courtes mais suffisantes pour permettre l'échange de larves entre populations à l'échelle démographique. (C) Ensemble de groupes discrets composant une seule et même population fermée ; la dispersion se produit à suffisamment longue distance pour que les groupes soient très connectées. NB : D'autres types de structures de populations existent (voir par exemple Freckleton and Watkinson (2002), Grimm et al. (2003)

ponctuellement élevé. Cela suggère que parmi les trois grands types de structure de population proposés par Kritzer and Sale (2004) (fig. 7.1), les populations de *D. aruanus* dans le SWL constitueraient bien une métapopulation (fig. 7.1B) plutôt qu'un ensemble de populations fermées aux dynamiques indépendantes (fig. 7.1A) ou qu'une seule et même population fragmentée mais composée de groupes très connectés (fig. 7.1C). L'efficacité de mesures de protection sur la persistance de cette métapopulation dans le SWL va donc fortement dépendre du choix des populations locales à protéger, choix qui devra prendre en compte la démographie de ces populations locales et les échanges

d'individus avec les autres populations. Une caractéristique qui distingue les métapopulations des populations composées de groupes discrets mais très connectés est une possible asynchronie dans la dynamique des sous-populations qui la composent (Sale et al. 2006), propriété qu'il conviendra d'étudier pour la demoiselle dans le SWL avant d'en évaluer les conséquences potentielles en termes de gestion. L'importance de la variabilité temporelle de l'auto-recrutement et du recrutement suggère une variabilité de la connectivité entre populations au sein du SWL. Les conséquences de ces fluctuations sur le fonctionnement des métapopulations et leur persistance sont encore peu connues et font l'objet de recherches très récentes (Watson et al. 2012, Williams and Hastings 2013, Snyder et al. 2014)). Watson et al. (2012) démontrent que ces fluctuations peuvent faire diminuer les taux de croissance au sein de la métapopulation en comparaison à des conditions non variables, en particulier pour des espèces à longue vie larvaire et qui ne se reproduisent qu'une fois pas an. Snyder et al. (2014) montrent que pour une espèce se reproduisant plusieurs fois par an (*Stegastes partitus*), dont l'écologie est proche de *D. aruanus*, ces fluctuations de connectivité ont très peu d'effet sur les taux de croissance de la métapopulation.

7.2 Perspectives

Une autre technique de marquage, couramment utilisée pour répondre aux questions de connectivité démographique à des échelles spatiales comparables à ce travail, est l'analyse génétique de parenté. L'avantage de cette autre technique est qu'elle permet d'identifier les parents des recrues collectées et donc de faire le lien entre la dispersion et les conditions parentales. Le marquage chimique transgénérationnel pourrait techniquement permettre d'identifier les parents de chaque individu mais cela nécessiterait d'injecter chaque adulte avec une marque différente (Huelga-Suarez et al. 2012) ce qui se révélerait coûteux et compliqué à mettre en oeuvre. Néanmoins les analyses de parenté nécessite d'échantillonner la population adulte de façon exhaustive afin de réaliser les tests statistiques de parenté (Sale et al. (2010) ; mais voir Christie et al. (2013)) alors que dans le cas du marquage transgénérationnel, la marque transmise par les femelles à leurs descendants est univoque (Thorrold et al. 2006). Ceci représente un avantage lorsque les populations à échantillonner sont grandes, ce qui est le cas des populations de *D. aruanus*, car il est alors possible de ne marquer qu'une fraction de la population adulte. De plus l'otolithe peut fournir de nombreuses informations complémentaires aux informations de marquage, telles que l'âge du poisson, sa croissance, sa durée de vie larvaire et même éventuellement sa trajectoire pendant la dispersion sous réserve de pouvoir utiliser la chimie naturelle. Les analyses de parenté peuvent quant à elles permettre d'étudier les relations de fraternité entre les individus (Beldade et al. 2012). Une utilisation en parallèle des deux techniques, si

elle est possible, permet d'une part la validation croisée de leurs résultats et d'autre part l'apport de connaissances complémentaires.

La résolution spatiale du modèle biophysique utilisé dans ce travail est relativement fine (maille carrée de 500 m de côté en horizontal et 30 niveaux verticaux). Néanmoins les différences entre modèle et données concernant les estimations d'auto-recrutement laissent penser que cette résolution pourrait ne pas être suffisamment précise autour des patchs de récifs pour prendre en compte les effets de circulation à très petite échelle, qui sont peut-être déterminants pour la rétention au niveau d'un récif. La mise en place d'un modèle à grille non structurée permettrait de faire varier la résolution spatiale, e.g., très fine autour des récifs et plus large dans des zones de pleine eau, sans augmenter le temps de simulation et le volume de stockage (Thomas et al. 2014). De plus notre modèle dans sa version actuelle ne prend pas en compte plusieurs paramètres tels que la migration verticale des larves, leur mortalité différentielle en fonction de leur population natale, ou encore la croissance des larves en lien par exemple avec la température et la disponibilité en nourriture qui sont des facteurs qui peuvent influencer significativement les valeurs de connectivité et d'auto-recrutement simulées par un modèle (Paris and Cowen 2004, Leis 2006).

Cette étude était limitée géographiquement au lagon sud-ouest de Nouvelle-Calédonie. L'inversion des courants due à la marée dans le canal de la Havannah rend les échanges larvaires entre cette partie du lagon et le lagon est très peu probables. Les résultats de Planes et al. (1998) vont d'ailleurs dans ce sens en montrant une différentiation génétique entre les populations du SWL et celles du lagon est pour deux autres espèces de poissons récifaux (*Stegastes nigricans* et *Acanthurus triostegus*). Par contre, des connexions sont possibles à l'échelle démographique entre le SWL et le lagon sud, puisque le SWL est essentiellement alimenté par les eaux qui entrent par le sud. Il serait donc intéressant dans les études futures d'élargir la zone d'étude au lagon sud. Des échanges possibles avec des populations hors lagon (e.g., îles Chesterfields) semblent peu probables à l'échelle démographique au vu des distances considérables entre ces populations et le SWL. Il serait cependant intéressant d'étudier les connexions par dispersion larvaire en intégrant l'échelle intra-lagonaire à l'échelle plus globale de la Mer de Corail, notamment dans le contexte actuel de création d'un parc naturel marin de la Mer de Corail initié par le gouvernement français.

Enfin, dans ce travail la dispersion a été étudiée du départ des larves à leur installation. On parle de connectivité potentielle (Pineda et al. 2007, Burgess et al. 2012). Or toutes les larves qui s'installent dans une population adulte à l'issue de leur vie pélagique ne survivront pas jusqu'à la reproduction. Pour pouvoir mesurer l'impact de la dispersion sur la démographie il est donc nécessaire de prendre en considération les

taux de survie post-installation dans l'estimation de la connectivité dite alors réalisée (Pineda et al. 2007). Cette survie peut elle aussi dépendre de multiples facteurs, y compris d'effets parentaux liés à la population natale (Nanninga 2013), et donc différer entre auto- et allo-recrues. Cette étape sera indispensable à l'intégration des résultats de cette étude dans une approche de dynamique de population plus complète.

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Communications

Articles scientifiques

Cuif M., D. M. Kaplan, J. Lefèvre, V. M. Faure, M. Caillaud, P. Verley, L. Vigliola, C. Lett. (2014) *Wind-induced variability in larval retention in a coral reef system : A biophysical modelling study in the South-West Lagoon of New Caledonia.* Progress in Oceanography 122, 105 - 115

Cuif M., F. Keller, O. Chateau, D. M. Kaplan, M. Labonne, C. Lett, L. Vigliola. (2014) *Evaluation of transgenerational isotope labeling of embryonic otoliths in a coral reef damselfish with single and repeated injections of enriched ^{137}Barium.* Journal of Experimental Marine Biology and Ecology 459, 151 - 159

Cuif M., D. M. Kaplan, C. Lett, L. Vigliola. (under review) *Monthly variability of self-recruitment for a coral reef damselfish.* Coral Reefs.

Lett C., T. Nguyen-Huu, **M. Cuif**, P. Saenz-Agudelo, D. M. Kaplan. (under review) *Linking local retention, self-recruitment and persistence in marine metapopulations.* Ecology.

Verley P., G. Andres, S. Bonhommeau, **M. Cuif**, L. Garavelli, B. Malauene, A.E. Nieblas, N. Putman, D. Kaplan, C. Lett, (submitted) *Ichthyop, a customizable tool for modeling ichthyoplankton dynamics.* Environmental Modelling & Software.

Congrès international

12th International Coral Reef Symposium. 9-13 Juillet 2012. Cairns, Queensland, Australia.
Cuif Marion, David Kaplan, Maylis Labonne, Christophe Lett, Laurent Vigliola. *Larval connectivity assessed with biophysical modelling and otolith transgenerational marking.* Theme : Larval ecology, recruitment & connectivitiy. Mini-symposium : The ecological importance of larval dispersal. Talk of 15 minutes.

Séminaires

RIEL seminar. 7 novembre 2013. Charles Darwin University, Darwin, Australia.
Cuif Marion, David Kaplan, Christophe Lett, Laurent Vigliola. *Larval connectivity assessed with biophysical modelling and otolith transgenerational marking.*

Jeudis d'EME. 6 février 2014. IRD, Sète, France.
Cuif Marion, David Kaplan, Christophe Lett, Laurent Vigliola. *Connectivité des popu-*

lations marines par dispersion larvaire. Approche par modélisation biophysique et marquages isotopiques.

Séminaire annuel d'UMMISCO. 11 mars 2014. IRD, Bondy, France.
Cuif Marion, David Kaplan, Christophe Lett, Laurent Vigliola. *Connectivité des populations marines par dispersion larvaire. Approche par modélisation biophysique et marquages isotopiques.*

Réunions de projet

1ère réunion du projet ANR COMPO. 24 octobre 2011. IRD, Nouméa, Nouvelle-Calédonie.
Cuif Marion, David Kaplan, Christophe Lett. *Modélisation de la dispersion des larves de Demoiselle (Dascyllus aruanus) dans le lagon Sud-Ouest de Nouvelle-Calédonie.*

1ère réunion du GDR MarCo. 7-10 novembre 2011. SMEL, Sète, France.
Kaplan David, **Marion Cuif**, Cécile Fauvelot, Pascal Dumas, Christophe Lett, Tri Nguyen-Huu, Josina Tiavouane, Laurent Vigliola. *Projet ANR COMPO : étude de la connectivité larvaire dans l'écosystème corallien de Nouvelle-Calédonie.*

2ème réunion du projet ANR COMPO. 3 septembre 2012. IRD, Sète, France.
Cuif Marion, David Kaplan, Christophe Lett, Laurent Vigliola. *Bilan de la 1ère année de thèse.*

2ème réunion du GDR MarCo. 8-11 octobre 2012. IFREMER, Roscoff, France.
Cuif Marion & Fauvelot Cécile, Pascal Dumas, David Kaplan, Christophe Lett, Tri Nguyen-Huu, Josina Tiavouane, Laurent Vigliola. *Projet ANR COMPO : étude de la connectivité larvaire dans l'écosystème corallien de Nouvelle-Calédonie.*

3ème réunion du projet ANR COMPO. 17 octobre 2013. IRD, Sète & Montpellier, France.
Cuif Marion, David Kaplan, Christophe Lett, Laurent Vigliola. *Bilan de la 2ème année de thèse.*

4ème réunion du GDR MarCo et du projet ANR COMPO. 2-6 juin 2014. IRD, Sète & Montpellier, France :

Cuif Marion, Christophe Lett, David Kaplan, Laurent Vigliola. *Marquage isotopique transgénérationnel des otolithes : expériences en aquarium et estimation de l'auto-recrutement*

d'une population de demoiselles (Dascyllus aruanus) dans le lagon sud-ouest de Nouvelle-Calédonie.

Saulnier Erwan, **Marion Cuif**, Christophe Lett, Laurent Vigliola. *Etude de la capacité reproductrice d'une population de demoiselles (Dascyllus aruanus) dans le lagon sud-ouest de Nouvelle-Calédonie.*

Doctoriales

Doctoriales 2011 de l'Université de Nouvelle-Calédonie. 13-14 octobre 2011. UNC, Nouméa, Nouvelle-Calédonie.
Cuif Marion, Christophe Lett, David Kaplan, Laurent Vigliola. *Connectivité des populations marines et implication pour la mise en place des réseaux d'aires marines protégées : application au Lagon sud-ouest de Nouvelle-Calédonie.*

Doctoriales 2012 de l'Université de Nouvelle-Calédonie. 8-9 octobre 2012. UNC, Nouméa, Nouvelle-Calédonie.
Cuif Marion, Christophe Lett, David Kaplan, Laurent Vigliola. *Connectivité des populations marines et implication pour la mise en place des réseaux d'aires marines protégées : application au Lagon sud-ouest de Nouvelle-Calédonie.*

Journée des formations doctorales IPEF. 30 janvier 2014. ENGREF, Paris, France.
Cuif Marion, Christophe Lett, David Kaplan, Laurent Vigliola. *Connectivité des populations marines par dispersion larvaire. Approche par modélisation biophysique et marquages isotopiques.*

Journée des doctorants et post-doctorants de la future UMR MARBEC. 19 juin 2014. IRD, Sète, France.
Cuif Marion & Tournois Jennifer. *Connectivité écologique des populations marines, études par microchimie des otolithes.*

Enseignement & encadrement

Modules d'enseignements EME, IRD, Sète, France :

- **Approche Ecosystémique des Ressources Marines Exploitées (AERME).** 3 décembre 2013.
  Cuif Marion, David Kaplan, Christophe Lett, Laurent Vigliola. *Connectivité larvaire des populations marines : Modélisation biophysique et marquages isotopiques des otolithes.*

- **Quantitative Ecosystem Approach to Fisheries (Q-EAF).** 18 avril 2014.
  Cuif Marion, David Kaplan, Christophe Lett, Laurent Vigliola. *Larval connectivity assessed with biophysical modelling and otolith transgenerational marking*.

Encadrement de stages de Master 1 :

- **Erwan Saulnier, Agrocampus Ouest.** Septembre 2012 - Février 2013.
  Evaluation de la capacité reproductrice d'une population de Dascyllus aruanus du lagon sud de Nouvelle Calédonie.
- **Mathis Vannereau, Université de La Rochelle.** Avril - Août 2013.
  Traits de vie du poisson demoiselle Dascyllus aruanus en Nouvelle-Calédonie par analyse optique des otolithes.

Médias

Canal IRD - Vidéo en ligne. Septembre 2013.
Cuif Marion. Réalisation : Jean-Michel Boré. Conseiller scientifique : Laurent Vigliola.
Connectivité des populations de poissons en Nouvelle-Calédonie.

Sciences au Sud. Juillet/Août 2014. *Les larves de poisson au bénéfice des vents*.

Investissement dans la vie étudiante

Mise en place et animation des petits déjeuners étudiants à l'IRD de Nouméa. 2011, 2012 & 2013.

Représentante des étudiants d'EME. 2013 & 2014 :

- Représentation des étudiants aux conseils d'unité.
- Organisation des mercredis étudiants.
- Préparation de l'évaluation AERES 2014.
- Organisation de la Journée des Doctorants et Post-Doctorants de la future UMR MARBEC du 19 juin 2014.

Membre actif de l'organisation du 10ème colloque étudiants "Ecology and Behaviour". 12-16 mai 2014. Montpellier, France. Recherche de financement & définition des thématiques de sessions.

Contributions

J'ai eu la chance de bénéficier au cours de ma thèse de l'aide technique de nombreuses personnes. Le tableau suivant a pour but de clarifier mon implication personnelle dans les diverses tâches techniques qu'ont nécessité l'expérience en aquarium, les marquages sur le terrain, les suivis biologiques et la modélisation biophysique. Je donne ici pour chaque tâche une estimation de ma contribution personnelle et le type de collaboration dont j'ai bénéficié pour leur réalisation.

Tâche technique	Contribution personnelle	Type de collaboration
Marquages : aquarium		
Capture colonies	100%	Aquarium des Lagons de Nouméa
Maintenance poissons	0%	Aquarium des Lagons de Nouméa
Injections	100%	Aquarium des Lagons de Nouméa
Suivi journalier pontes	20%	Aquarium des Lagons de Nouméa
Elevage larves	20%	Aquarium des Lagons de Nouméa
Mesures oeufs et larves	100%	
Extraction et préparation otolithes	100%	
Analyse LA-ICP-MS	100%	LEMAR Brest, Charles Darwin University
Marquages : terrain		
Injections et collectes en plongée	100%	Plongeurs COREUS
Extraction otolithes	85%	Techniciens COREUS
Préparation otolithes	70%	LEMAR Brest, techniciens COREUS
Analyse LA-ICP-MS	100%	Charles Darwin University
Suivi du recrutement	50%	Plongeurs COREUS
Etude Reproduction		
Encadrement stage	50%	Laurent Vigliola, Yves Letourneur
Collecte gonades en plongée	100%	
Extraction gonades	100%	
Préparation, analyses gonades	5%	Stagiaire M2
Etude PLD		
Encadrement stage	50%	Laurent Vigliola, Maylis Labonne
Préparation otolithes	50%	Stagiaire M1
Lectures	70%	Stagiaire M1
Modèle biophysique		
Préparations sorties MASR3D	0%	IFREMER
Création logiciel Ichthyop	0%	Ingénieur EME
Simulations et post processing	100%	

Remerciements

Et voilà trois années intenses qui se terminent. Entre Sète et Nouméa, avec quelques passages à Brest et Darwin, ces trois années furent riches en rencontres et je voudrais remercier toutes les personnes qui m'ont entourée.

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